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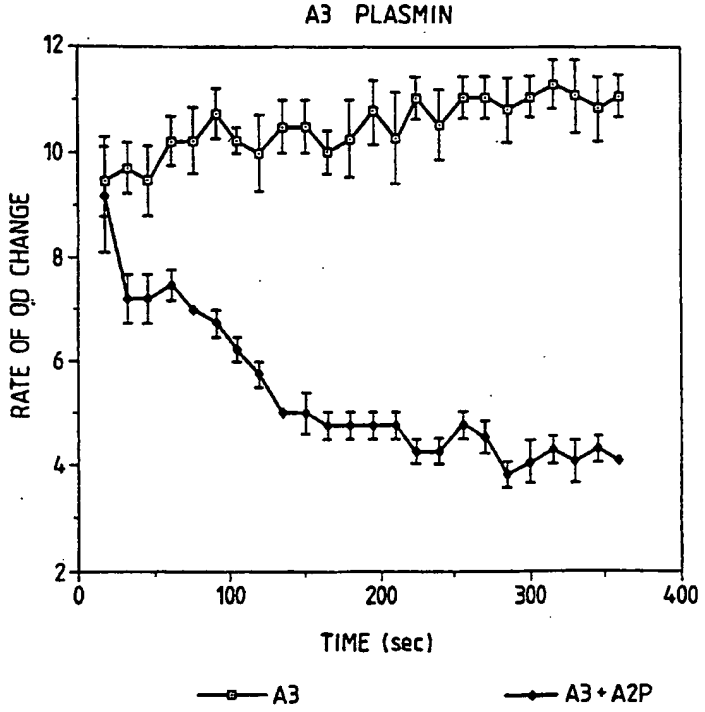

(57) Abstract

Serine proteases of the chymotrypsin superfamily are modified so that they exhibit resistance to serine protease inhibitors. If such modified serine proteases have fibrinolytic, thrombolytic, antithrombotic or prothrombotic properties, they are useful in the treatment of blood clotting diseases or conditions.

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INHIBITOR RESISTANT SERINE PROTEASES

The present invention relates to serine proteases of the chymotrypsin superfamily which have been modified so that they exhibit resistance to serine protease inhibitors. The invention also relates to the precursors of such compounds, their preparation, to nucleic acid coding for them and to their pharmaceutical use.

Serine proteases are endopeptidases which use serine as the nucleophile in peptide bond cleavage. There are two known superfamilies of serine proteases and these are the chymotrypsin superfamily and the Streptomyces subtilisin superfamily (Barrett, A.J., in: Proteinase Inhibitors, Ed. Barrett, A.J. *et al.*, Elsevier, Amsterdam, pp 3-22 (1986) and James, M.N.G., in: Proteolysis and Physiological Regulation, Ed. Ribbons, D.W. et al, Academic Press, New York, pp 125-142 (1976)).

The present invention is particularly concerned with serine proteases of the chymotrypsin superfamily which includes such compounds as plasmin, tissue plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), trypsin, chymotrypsin, granzyme, elastase, acrosin, tonin, myeloblastin, prostate-specific antigen (PSA), gamma-renin, tryptase, snake venom serine proteases, adipsin, protein C, cathepsin G, complement components C1R, C1S and C2, complement factors B, D and I, chymase, hepsin, medullasin and proteins of the blood coagulation cascade including kallikrein, thrombin, and Factors VIIa, IXa, Xa, XIa and XIIa. Members of the chymotrypsin superfamily have amino acid and structural homology of the catalytic domains, although a comparison of the sequences of the catalytic domains reveals the presence of insertions or deletions of amino acids.

However, these insertions and deletions map to the surface of the folded molecule and thus do not affect the basic structure although it is likely that they contribute to the specificity of interactions of the molecule with substrates and inhibitors (Strassburger, W. et al, FEBS Lett., **157**, 219-223 (1983)).

Serine protease inhibitors are also well known and are divided into the following families: the bovine pancreatic trypsin inhibitor (BPTI) family, the Kazal family, the alpha-2-macroglobulin (A2M) family, the Streptomyces subtilisin inhibitor (SSI) family, the serpin family, the Kunitz family, the four-disulphide core family, the potato inhibitor family and the Bowman-Birk family.

Serine protease inhibitors inhibit their cognate serine proteases and form stable 1:1 complexes with these proteases. Structural data are available for several protease-inhibitor complexes including trypsin-BPTI, chymotrypsin-ovomucoid inhibitor and chymotrypsin-potato inhibitor (Read, R.J. et al., in: Proteinase Inhibitors, Ed. Barrett, A.J. et al., Elsevier, Amsterdam, pp 301-336 (1986)). A structural feature which is common to all the serine protease inhibitors is a loop extending from the surface of the molecule which contains the recognition sequence for the active site of the cognate serine protease and, in fact, there is remarkable similarity in the specific interactions between different inhibitors and their cognate serine proteases, despite the diverse sequences of the inhibitors.

The serine proteases of the chymotrypsin superfamily play an important role in human and animal physiology. Some of the most important serine protease inhibitors are

those which are involved in blood coagulation and fibrinolysis. In the process of blood coagulation, a cascade of enzyme activities is involved in generating a fibrin network which forms the framework of a clot or thrombus. Degradation of the fibrin network (fibrinolysis) involves the protease inhibitor plasmin. Plasmin is formed in the body from its inactive precursor plasminogen by cleavage of the peptide bond between arginine 561 and valine 562 of plasminogen. This reaction is catalysed by t-PA or by u-PA.

If the balance between the clotting and fibrinolytic systems becomes locally disturbed, intravascular clots may form at inappropriate locations leading to conditions such as coronary thrombosis and myocardial infarction, deep vein thrombosis, stroke, peripheral arterial occlusion and embolism. A known way of treating such conditions is to administer to a patient a serine protease of the chymotrypsin superfamily or the precursor of such an enzyme. For example, t-PA, u-PA and plasminogen in the form of anisoylated plasminogen complexed with streptokinase are used in the treatment of myocardial infarction; plasminogen is used to supplement the natural circulatory plasminogen level to enhance thrombolytic therapy; and protein C is used as an antithrombotic agent. Serine proteases of the chymotrypsin superfamily, for example factors VIIa and IX, are administered for induction of blood clotting in disorders such as haemophilia. A major problem with the use of all of these agents in this type of therapy is their rapid neutralisation by serine protease inhibitors which reduces the efficiency of the therapy and increases the dose of agent required. It would therefore be advantageous to develop modified analogues of these endopeptidases which are resistant to inactivation by

serine protease inhibitors whilst maintaining their activity. However, it is not easy to predict modifications which will result in increased resistance to inhibition without significant decrease in endopeptidase activity.

WO-A-9010649 discloses serine proteases of the chymotrypsin superfamily which have been modified and which are said to have increased resistance to serine protease inhibitors. The authors of that document have studied the known structure of the complex between trypsin and BPTI and have realised that, other than the amino acids in the major recognition site, the amino acids of trypsin that make direct contact with BPTI are located in the region between residues 37 and 41 and in the region between residues 210 to 213 of the polypeptide chain. The authors have then extrapolated from this on the basis that there is a high degree of structural homology between the catalytic domains of serine proteases and have suggested that mutation of a residue in any serine protease equivalent to the Tyr-39 residue in trypsin would lead to increased resistance of the modified analogue compared with the wild-type serine protease. They also suggest that inhibition resistant t-PA analogues can be made by mutation of an additional stretch of seven amino acids which occurs in tPA, but not in trypsin, adjacent to the predicted contact point at Arg-304 (equivalent to Tyr-39 of trypsin). However, although the catalytic domains of members of the chymotrypsin superfamily of serine proteases do, in general, have sequence and structural homology, Tyr-39 of trypsin is on a loop structure on the surface of the protein and, as is shown in Figure 1, the equivalent regions of other serine proteases are highly variable within the superfamily. Indeed, this is acknowledged in

WO-A-9010649. It is, therefore, by no means evident that the specific conformation of the loop in this region of the protein is conserved between different serine proteases, especially in cases where the number of residues in the loop differ, as is the case for trypsin and plasmin. Thus, although the residues in the region may be aligned sequentially because of the alignment of their flanking regions which do have similar sequences, it is not at all evident that their side-chains are in equivalent spacial locations and, therefore, residues which are equivalent in a sequence alignment are not necessarily able to form equivalent interactions in the folded protein. If plasmin is taken as an example, it can be seen from Figure 1 that there are three hydrophobic residues (Phe-22, Met-24 and Phe-26) which could be involved in a similar hydrophobic interaction to that of Tyr-39 in the trypsin/BPTI complex. The numbering of the plasmin residues just mentioned is the numbering of SEQ ID No 2 which depicts the protease domain of plasmin. The residue designated 1 in SEQ ID No 2 is at position 562 of the mature protein. A study of Figure 1 shows that any of these residues could be equivalent to Tyr-39 of trypsin which occurs at position 29 in the numbering system of Figure 1. Clearly, therefore, the method described in WO-A-9010649 for designing a protease which is resistant to inhibition is not wholly reliable and it would be preferable to design inhibition resistant mutants in a different way.

The present inventors have realised that, because the serine protease inhibitors are structurally homologous in their active centre loop and form similar interactions with their cognate serine proteases (Read, R.J. *et al.*, in: Proteinase Inhibitors, Ed. Barrett, A.J. *et al.*, Elsevier, Amsterdam, pp 301-336 (1986)), mutations in any

given serine protease which result in resistance to inhibition by a serine protease inhibitor may be applicable to mutations of spatially or sequentially equivalent residues in any other member of the chymotrypsin superfamily.

The interaction between enzyme and inhibitor responsible for inhibition of enzyme activity involves the catalytic site amino acids of the enzyme and the reactive site amino acids of the inhibitor. This principal interaction is stabilised by other interactions between the molecules. Although there is a comparatively large surface of interaction between the protease and the inhibitor, the protease/inhibitor complex is mainly stabilised by a few key interactions. These are exemplified by the interactions observed in the protease/inhibitor complex between trypsin and BPTI (Huber, R. et al., *J. Mol. Biol.* **89**:73-101 (1974)), which serves as a model for the interaction between the catalytic domains of other serine proteases and their cognate inhibitors. In the trypsin/BPTI complex, the key residues of the protease, apart from those in the principal recognition site, which interact with the inhibitor are residues 37-41 and 210-213 (chymotrypsin numbering), with Tyr-39 being the most important. This interaction served as the basis for WO-A-9010649 in which the spatially equivalent residues in the t-PA/PAI-1 complex were identified, and inhibitor-resistant mutants were described.

In contrast to the disclosure WO-A-9010649, the present inventors have realised that the desired disruption of the protease/inhibitor interactions which lead to inhibitor resistance need not be caused by mutating the specific residues identified in that document or their equivalents in other serine proteases. Instead, residues

in spacial, rather than sequential, proximity to these key residues, may be mutated resulting in a less stable complex between the protease and the inhibitor.

In a first aspect of the present invention, there is provided a modified endopeptidase of the chymotrypsin superfamily of serine proteases or a precursor of such an endopeptidase, which is resistant to serine protease inhibitors, characterised in that the modification comprises the mutation of one or more residues in close spacial proximity (other than sequential proximity) to a site of interaction between the protease and a cognate protease inhibitor.

In the context of this invention, the term 'precursor', when used in relation to a serine protease, refers to a protein which is cleavable by an enzyme to produce an active serine protease.

Mutations resulting in resistance to the inhibitor may induce:

i) a conformational change in the local fold of the protease such that the resulting complex with the inhibitor is less stable than the equivalent complex between the inhibitor and the wild-type protein;

ii) a change in the relative orientations of the protease and inhibitor on forming a complex such that the resulting complex is less stable than the equivalent complex between the inhibitor and the wild-type protein;

iii) a change in the steric bulk of the protease in

the region of the inhibitor-binding site such that the resulting complex is less stable than the equivalent complex between the inhibitor and the wild-type protein;

iv) a change in the electrostatic potential field in the region of the inhibtor-binding site such that the resulting complex is less stable than the equivalent complex between the inhibitor and the wild-type protein; or

v) any combination of the above.

The residues to be mutated need not be sequentially close to the key residues involved in the protease/inhibitor interaction, since the three-dimensional folding of the protease chain brings sequentially distant residues into spatial proximity. It is necessary to select the residues for mutation based on a model of either the protease used to generate the mutant, or of another member of the chymotrypsin superfamily of serine proteases. Where the three-dimensional structure of the protease to be mutated is not known, the selection of residues for mutation may be based either on a three-dimensional model of the protein to be mutated derived using homology modelling or other techniques, or on sequence alignments between the protein to be mutated and other members of the chymotrypsin superfamily of serine proteases with known three-dimensional structures. If sequence alignments are employed, it is not necessary to generate a three-dimensional structural model of the protease of interest in order to select residues for mutation to give inhibitor resistance, as spatial proximity to the key residues can be inferred from those proteins in the alignment with known three-dimensional structures. The

spatial relationships between the residues to be mutated and the key residues in the protease/inhibitor interaction may be inferred by any appropriate method. Suitable methods are known to those skilled in the art.

The modified serine protease may be any serine protease of the chymotrypsin superfamily since all of these enzymes have a common mechanism of action. Examples of serine protease inhibitors which can be modified according to the present invention are as follows:

plasmin, tissue plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), trypsin, chymotrypsin, granzyme, elastase, acrosin, tonin, myeloblastin, prostate-specific antigen (PSA), gamma-renin, tryptase, snake venom serine proteases, adipsin, protein C, cathepsin G, complement components C1R, C1S and C2, complement factors B, D and I, chymase, hepsin, medullasin and proteins of the blood coagulation cascade including kallikrein, thrombin, and Factors VIIa, IXa, Xa, XIa and XIIa.

However, modified analogues of plasmin, t-PA, u-PA, activated protein C, thrombin, factor VIIa, factor IXa, factor Xa, factor XIa and factor XIIa are particularly useful, as is a modified version of plasminogen, since all of these compounds can be used as fibrinolytic or thrombotic agents. An inhibition resistant plasmin analogue is particularly preferred.

The serine protease inhibitor to which the modified serine protease of the invention is resistant will obviously depend on which serine protease has been modified. In the case of plasmin, the primary physiological inhibitor is α2-antiplasmin which belongs

to the serpin family of serine protease inhibitors. The reaction between plasmin and α2-antiplasmin consists of two steps: a very fast reversible reaction between the kringle 1 lysine binding site of plasmin and the carboxy-terminal region of the inhibitor, followed by a reaction between the catalytic site of plasmin and the reactive site of the inhibitor which results in the formation of a very stable 1:1 stoichiometric enzymatically inactive complex (Holmes, W.E. et al., J. Biol. Chem., **262**, 1659-1664 (1987)). Therefore, when the serine protease is plasmin, it is particularly useful if the serine protease inhibitor to which the plasmin is resistant is α2-antiplasmin. Plasmin is also inhibited by α2-macroglobulin and α1-antitrypsin and resistance to inhibition by these inhibitors is also useful.

From a three-dimensional model of the plasmin/antiplasmin complex, (described in Method 1), it has been determined that, in plasmin, the residues which are in close spatial proximity to the key residues of interaction between the protease and the inhibitor are residues 17-20, 44-54, 62, 154, 158, 198-213. The numbering used above is the numbering system of sequence ID No 2 which represents the protease domain of plasmin and begins at position 562 of the mature protein. In order to be resistant to inhibition by a serine protease inhibitor such as α2-antiplasmin, it is necessary to modify plasmin in one or more of these regions. Protease inhibition resistance can be induced in other serine proteases of the chymotrypsin superfamily by modifying equivalent regions of these proteins. Figure 1 shows the sequences of the protease domains of a variety of proteases and, from a study of Figure 1, it is clear

where modifications should be made in order to induce resistance to protease inhibitors. In the numbering system of Figure 1, the modification regions just mentioned occur at residues 17-22, 49-64, 72, 203, 214, and 264-281. The types of mutations which are suitable for inducing resistance to inhibition include single or multiple amino acid substitutions, additions or deletions. However, amino acid substitutions are particularly preferred.

In plasmin, examples of amino acid substitution mutations which result in a modified response to inhibition by α2-antiplasmin, using the numbering system of SEQ ID No 2, are Glu-62 to Lys or Ala, Ser-17 to Leu, Arg-19 to Glu or Ala, and Glu-45 to Lys, Arg or Ala. Resistance to protease inhibition can be induced in other serine proteases by making modifications at equivalent positions. The degree of resistance to inhibition may be altered by making either single or multiple mutations in the protease, or by altering the nature of the amino acid used for substitution.

In addition to the modification of the invention, the serine protease may be modified in other ways as compared to wild-type proteins. Any modifications may be made to the protein provided that it does not lose its activity.

As an alternative to a modified serine protease, it is also possible to modify a precursor of the enzyme so that the enzyme derived from the precursor will have the desired resistance to inhibition. An example of a serine protease precursor is plasminogen which is the inactive precursor of plasmin. Conversion of plasminogen to plasmin is accomplished by cleavage of the peptide bond

between arginine 561 and valine 562 of plasminogen. Under physiological conditions this cleavage is catalysed by t-PA or u-PA. Cleavage of a modified plasminogen variant of the present invention will produce a plasmin variant as descried above and it is, of course, preferable that the plasminogen variant will be cleaved to produce one of the preferred plasmin variants described above.

Again, as with serine proteases, the precursors may have other modifications. Analysis of the wild-type plasminogen molecule has revealed that it is a glycoprotein composed of a serine protease domain, five kringle domains and an N-terminal sequence of 78 amino acids which may be removed by plasmin cleavage. Cleavage by plasmin involves hydrolysis of the Arg(68)-Met(69), Lys(77)-Lys(78) or Lys(78)-Val(79) bonds to create forms of plasminogen with an N-terminal methionine, lysine or valine residue, all of which are commonly designated as lys-plasminogen. Intact plasminogen is referred to as glu-plasminogen because it has an N-terminal glutamic acid residue. Glycosylation occurs on residues Asn(289) and Thr(346) but the extent and composition are variable, leading to the presence of a number of different molecular weight forms of plasminogen in the plasma. Any of the above plasminogen variants may be modified to produce a variant according to the present invention. The protein sequencing studies of Sottrup-Jensen et al (in: Atlas of Protein Sequence and Structure (Dayhoff, M.O., ed.) 5 suppl. 3, p.95 (1978)) indicated that plasminogen was a 790 amino acid protein and that the site of cleavage was the Arg(560)-Val(561) peptide bond. A plasminogen variant which is suitable for modification according to the present invention is a 791 residue protein with an extra Ile at position 65 and encoded by

cDNA isolated by Forsgren et al (FEBS Letters, **213**, 254-260 (1987)). The serine protease domain of any of these plasminogen analogues can be recognised by its homology with serine proteases and on activation to plasmin is the catalytically active domain involved in fibrin degradation. The five kringle domains are homologous to those in other plasma proteins such as tPA and prothrombin and are involved in fibrin binding and thus localisation of plasminogen and plasmin to thrombi.

The plasminogen analogues of the present invention may also contain other modifications (as compared to wild-type glu-plasminogen) which may be one or more additions, deletions or substitutions. Examples of particularly suitable plasminogen analogues are disclosed in our copending applications WO-A-9109118 and GB 9222758.6 and comprise plasminogen analogues which are cleavable by an enzyme involved in blood clotting to produce active plasmin. These plasminogen analogues may, according to the present invention, be further modified so that, on cleavage, the plasmin which is produced is resistant to inhibition by serine protease inhibitors such as α2-antiplasmin. Other plasminogen analogues which may be modified to produce the plasminogen analogues of the invention are analogues in which there has been an addition, removal, substitution or alteration of one or more kringle domains. Other suitable plasminogen analogues are Lys-plasminogen variants in which the amino terminal 68, 77 or 78 amino acids have been deleted. Such variants may have enhanced fibrin binding activity as has been observed for lys-plasminogen compared to wild-type glu-plasminogen (Bok, R. A. and Mangel, W. F., Biochemistry, **24**, 3279-3286 (1985)). Also included within the scope of the invention are plurally-modified

plasminogen analogues which include one or more modifications to prevent, reduce or alter glycosylation patterns. Such analogues may have a longer half-life, reduced plasma clearance and/or higher specific activity.

The modified serine proteases and serine protease precursors of the invention can be prepared by any suitable method and, in a second aspect of the invention, there is provided a process for the preparation of such a serine protease or serine protease precursor, the process comprising coupling together successive amino acid residues and/or ligating oligopeptides. Although the proteins may, in principle, be synthesised wholly or partly by chemical means, it is preferred to prepare them by ribosomal translation, preferably in vivo, of a corresponding nucleic acid sequence. The process may further include an appropriate glycosylation step.

It is preferred to produce proteins of the invention using recombinant DNA technology. DNA encoding a naturally occurring serine protease or precursor may be obtained from a cDNA or genomic clone or may be synthesised. Amino acid substitutions, additions or deletions are preferably introduced by site-specific mutagenesis. DNA sequences encoding glu-plasminogen, lys-plasminogen, other plasminogen analogues and serine protease variants may be obtained by procedures familiar to those skilled in the art of genetic engineering.

The process for producing proteins using recombinant DNA technology will usually include the steps of inserting a suitable coding sequence into an expression vector and transfecting the vector into a suitable host cell.
Therefore, in a third aspect of the invention there is provided nucleic acid coding for a modified serine

protease as described above. The nucleic acid may be either DNA or RNA and may be in the form of a vector such as a plasmid, cosmid or phage. The vector may be adapted to transfect or transform prokaryotic cells, such as bacterial cells and/or eukaryotic cells, such as yeast or mammalian cells. The vector may be a cloning vector or an expression vector and comprises a cloning site and, preferably, at least one marker gene. An expression vector will additionally have a promoter operatively linked to the sequence to be inserted into the cloning in site and, preferably, a sequence enabling the protein product to be secreted.

Most of the proteins of the present invention, including molecules such as tPA, can easily be obtained by inserting the coding sequence into an expression vector as described and transfecting the vector into a suitable host cell which may be a bacterium such as E. coli, a eukaryotic microorganism such as yeast or a higher eukaryotic cell. With molecules such as plasminogen which are unusually difficult to express, it may be necessary to use a vector of the type described in our copending application, WO-A-9109118, which comprises a first nucleic acid sequence coding for the modified serine protease, operatively linked to a second nucleic acid sequence containing a strong promoter and enhancer sequence derived from human cytomegalovirus, a third nucleic acid sequence encoding a polyadenylation sequence derived from SV40 and a fourth nucleic acid sequence coding for a selectable marker expressed from an SV40 promoter and having an additional SV40 polyadenylation signal at the 3' end of the selectable marker sequence. Such a vector may either comprise a single nucleic acid molecule or a plurality of such molecules so that, for example, the first, second and third sequences may be

contained in a first nucleic acid molecule and the fourth sequence may be contained in a second nucleic acid molecule. This vector is particularly useful for the expression of plasminogen and plasminogen analogues.

For any of the proteins of the invention, the vector is preferably chosen so that the protein is expressed and secreted into the cell culture medium in a biologically active form without the need for any additional biological or chemical procedures. In the case of plasminogen, this can be achieved using the vector described above.

In a further aspect of the invention there is provided a process for the preparation of nucleic acid encoding a modified serine protease which exhibits resistance to serine protease inhibitors, the process comprising coupling together successive nucleotides and/or ligating oligo- and/or poly-nucleotides.

In a further aspect of the invention, there is provided a cell transformed or transfected by a vector as described above. Suitable cells or cell lines include both prokaryotic and eukaryotic cells. A typical example of a eukaryotic cell is a bacterial cell such as E. coli. Suitable eukaryotic cells include yeast cells such as Sacchromyces cerevisiae or Pichia pastoris. Other examples of suitable eukaryotic cells are mammalian cells which grow in continuous culture and examples of such cells include Chinese hamster ovary (CHO) cells, mouse myeloma cell lines such as P3X63-Ag8.653 and NS0, COS cells, HeLa cells, 293 cells, BHK cells, melanoma cell lines such as the Bowes cell line, mouse L cells, human hepatoma cell lines such as HepG2, mouse fibroblasts and mouse NIH 3T3 cells. CHO cells are particularly suitable

as hosts for the expression of plasminogen and plasminogen analogues. The transformation of the cells may be achieved by any convenient method but electroporation is a particularly suitable method.

For some molecules, such as plasminogen, there may be a low level of undesirable activation during culture. Therefore, in a further aspect of the invention, there is provided a eukaryotic host cell transfected or transformed with a first DNA sequence encoding a serpin-resistant serine protease and with an additional DNA sequence encoding the cognate inhibitor.

The modified serine proteases of the present invention have a variety of uses and, if the serine protease is a fibrinolytic or thrombolytic enzyme, it will be useful in a method for the treatment and/or prophylaxis of diseases or conditions caused by blood clotting, the method comprising administering to a patient an effective amount of the serine protease.

Therefore, in a further aspect of the invention, there is provided a modified serine protease according to the first aspect of the invention, which is a serine protease having fibrinolytic, thrombolytic, antithrombotic or prothrombotic properties, for use in medicine, particularly in the treatment of diseases mediated by blood clotting. Such conditions include myocardial and cerebral infarction, arterial and venous thrombosis, thromboembolism, post-surgical adhesions, thrombophlebitis and diabetic vasculopathies.

The invention also provides the use of a modified fibrinolytic, thrombolytic, antithrombotic or prothrombotic serine protease according to the first

aspect of the invention in the preparation of an agent for the treatment and/or prophylaxis of diseases or conditions mediated by blood clotting. Examples of such conditions are mentioned above.

Furthermore, there is also provided a pharmaceutical or veterinary composition comprising one or more modified serine proteases of the first aspect of the invention together with a pharmaceutically and/or veterinarily acceptable carrier.

The composition may be adapted for administration by oral, topical or parenteral routes including intravenous or intramuscular injection or infusion. Suitable injectable compositions may comprise a preparation of the compound in isotonic physiological saline and/or buffer and may also include a local anaesthetic to alleviate the pain of the injection. Similar compositors may be used for infusions. If the compound is administered topically, it may be formulated as a cream, ointment or lotion in a suitable base.

The compounds of the invention may be supplied in unit dosage form, for example as a dry powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachet.

The quantity of material to be administered will depend on the amount of fibrinolysis or inhibition of clotting required, the required speed of action, the seriousness of the thromboembolic position and the size of the clot. The precise dose to be administered will, because of the very nature of the condition which compounds of the invention are intended to treat, be determined by the physician. As a guideline, however, a patient being

treated for a mature thrombus will generally receive a daily dose of a plasminogen analogue of from 0.01 to 10 mg/kg of body weight either by injection in for example up to 5 doses or by infusion.

The invention will now be further described by way of example only with reference to the following drawings in which:

Figure 1 shows the alignment of the catalytic domain amino acids of the chymotrypsin superfamily;

Figure 2 shows maps of the pGWH and pGWHgP vectors;

Figure 3 shows the effect of α2-antiplasmin on the activity of plasminogen mutant A3.

Figure 4 shows the sequence alignment of ovalbumin and α2-antiplasmin used to generate the α2-antiplasmin model.

The following examples further illustrate the invention. Examples 1 to 5 describe the expression of various plasminogen analogues from higher eukaryotic cells and example 6 describes an assay used to assess resistance to α2-antiplasmin.

Example 1 - Construction and Expression of A1 and A12

The isolation of plasminogen cDNA and construction of the vectors pGWH and pGWHgP (Figure 2) have been described in WO-A-9109118. In pGWHgP, transcription through the plasminogen cDNA can initiate at the HCMV promoter/ enhancer and the selectable marker gpt is employed.

The techniques of genetic manipulation, expression and protein purification used in the manufacture of the modified plasminogen examples to follow, are well known to those skilled in the art of genetic engineering. A description of most of the techniques can be found in one of the following laboratory manuals: "Molecular Cloning" by T. Maniatis, E.F. Fritsch and J. Sambrook published by Cold Spring Harbor Laboratory, Box 100, New York, or "Basic Methods in Molecular Biology" by L.G. Davis, M.D. Dibner and J.F. Battey published by Elsevier Science publishing Co Inc, New York.

Additional and modified methodologies are detailed in the methods section below.

Plasminogen analogues have been constructed which are designed to be resistant to inhibition by α2-antiplasmin. A1 is a plasminogen analogue in which the amino acid Phe-587 is replaced by Asn. A12 is a plasminogen analogue in which the Arg-580 is replaced by Glu. The modification strategy in this example is essentially as described in WO-A-9109118 Example 3, with the mutagenesis reaction carried out on the 1.87kb KpnI to HincII fragment of the thrombin activatable plasminogen analogue T19 cloned into the bacteriophage M13mp18. Single stranded template was prepared and the mutation made by oligonucleotide

directed mutagenesis. For A1, a 24 base long oligonucleotide 5'GGTGCCTCCACAATTGTGCATTCC3' (SEQ.ID. 3) was used to direct the mutagenesis and for A12 a 27 base oligonucleotide was used 5'CCAAACCTTGTTTCAAGACTGACTTGC 3' (SEQ ID 7).

Plasmid DNA was introduced into CHO cells by electroporation using 800 V and 25 µF as described in the methods section below. Selective medium (250 µl/ml xanthine, 5 µg/ml mycophenolic acid, 1x hypoxanthine-thymidine (HT)) was added to the cells 24 hours post transfection and the media changed every two to three days. Plates yielding gpt-resistant colonies were screened for plasminogen production using an ELISA assay. Cells producing the highest levels of antigen were re-cloned and the best producers scaled up into flasks with production being carefully monitored. Frozen stocks of all these cell lines were laid down. Producer cells were scaled up into roller bottles to provide conditioned medium from which plasminogen protein was purified using lysine SEPHAROSE 4B. (The word SEPHAROSE is a trade mark.)

Example 2 - Construction and Expression of A3 and A16

The procedure of Example 1 was generally followed except that the mutagenesis was performed on an EcoRV to HindIII fragment (0.85kb) containing the 3'of wild type plasminogen cloned into M13. The oligonucleotide used was a 27mer 5'GTTCGAGATTCACTTTTTGGTGTGCAC3' (SEQ.ID. 4) which changed Glu-623 to Lys, thus changing an acidic amino acid to a basic amino acid. The resulting mutant was cloned as an EcoRV to Sph1 fragment replacing the corresponding wild type sequence. The 27 base oligonucleotide 5'GTTCGAGATTCACTGCTTGGTGTGCAC3' (SEQ ID 10) was used to change Glu-623 to Ala to produce A16.

Example 3- Construction and Expression of A4, A14 and A15

Mutant A4 is designed to disrupt ionic interactions on the surface of plasminogen preventing binding to antiplasmin. The mutagenesis and sub-cloning strategy was as described in Example 1 using a 24 base oligonucleotide 5'CTTGGGGACTTCTTCAAGCAGTGG3' (SEQ.ID. 5) designed to convert Glu-606 to Lys. The 24 base oligonucleotide 5'CTTGGGGACTTGGCTAGACAGTGG 3' (SEQ ID 8) was used to change Glu-606 to Ala to produce A14 and the 25 base oligonucleotide 5'CTTGGGGACTTCCTTAGACAGTGGG 3' (SEQ ID 9) was used to change Glu-606 to Arg to produce A15.

Example 4 - Construction and Expression of A5

Plasminogen analogue A5 was designed to alter the positioning of the Tyr 39 containing structural loop and was made generally as described in the procedure of Example 1. In A5, Ser-578 has been replaced by Leu using the 24mer 5'CTCGTACGAAGCAGGACTTGCCAG3' (SEQ.ID. 6) on the KpnI to EcoRV fragment of plasminogen in M13 as the template. The mutation was cloned directly into pGW1Hg.plasminogen using the restriction enzymes HindIII and SplI. These sites had previously been introduced at the extreme 5' end of plasminogen and at 1850 respectively via mutagenesis; the plasminogen coding sequence was not affected by this procedure.

Example 5 - Construction and Expression of double mutant A3A4

Plasminogen mutant A3A4 combines the two mutations A3 and A4 as described in Examples 2 and 3 respectively.

Mutagenesis was performed on the EcoRV to SphI fragment of A4 cloned into M13 using the A3 mutagenesis oligonucleotide (SEQ ID4).

Example 6 - Plasmin-Antiplasmin Interaction Assays

A chromogenic assay was used to assess the resistance of the plasmin(ogen) mutants to inhibition by α2-antiplasmin. Inhibition of plasmin activity was determined by the change in the rate of cleavage of the plasmin chromogenic substrate S2251 (Quadratech, P.O.Box 167, Epsom, Surrey. KT17 2SB).

Prior to assay, the plasminogens were activated to plasmin using either urokinase for mutants in wild type plasminogen, or thrombin for thrombin activatable plasminogen mutants (WO-A-9109118). Activation of wild-type plasminogen to plasmin was achieved by incubation of the plasminogen (ca. 14 µg) with urokinase (16.8×10^{-3} U) in 1750 µl of assay buffer (50 mM Tris, 0.1 mM EDTA, 0.00005% Triton X100, 0.1% (w/v) human serum albumin, pH 8.0) at 37°C for 5 mins. Activation of thrombin activatable plasminogen mutants to plasmin was achieved by incubation of the plasminogen (ca. 14 µg) with thrombin in 1750 µl of assay buffer at 37°C. Hirudin was added to inhibit the thrombin activity as thrombin cleaves the chromogenic substrate.

Plasmin (125µl) was mixed with 250 µl S2251 (2 mg/ml in assay buffer) and 125 µl antiplasmin (1.25 µg in assay buffer, #4032 American Diagnostica Inc., 222 Railroad Avenue, P.O.Box 1165, Greennwich, CT06836-1165) or 125 µl assay buffer in a cuvette and the absorbance at 405nM measured over time.

A Beckman DU64 spectrophotometer and Beckman "Data Leader" data capture software were used to record absorbance at 405nM at 1 sec intervals for 8 minutes. The Data Leader software package was used to calculate the first derivative of the data to provide the rate of change of absorbance at 405nM against time, an estimate of active plasmin concentration against time. Wild type plasmin was rapidly inactivated by α2-antiplasmin; after only 15 seconds the plasmin was essentially inactivated. In contrast, plasminogen mutant A3 has an antiplasmin resistant phenotype and is only slowly inactivated by α2-antiplasmin with a t1/2 (half the rate of OD change at t=15 sec) of approximately 75 seconds (Figure 3).

METHODS

1.Model structures were built by homology based on the x-ray structures of trypsin/BPTI. A refined plasminogen structure was modelled by homology to thrombin using the PPACK/thrombin x-ray structure from Bode et al. (Bode, W. et al., **EMBO J.** 8:3467-3475 (1989). A refined alpha-2-antiplasmin [A2AP] structure was modelled by homology to ovalbumin using atomic co-ordinates from the Brookhaven Protein Data Bank entry 1OVA, except for the loop containing the reactive bond, which was modelled using the co-ordinates for residues 13 to 19 of BPTI from the PDB entry 2PTC. The alignment used to generate the A2AP model is shown in Figure 4. The A2AP model described here does not include co-ordinates for the 79 N-terminal residues and 55 C-terminal residues.

Most serine-protease-directed inhibitors react with cognate enzymes according to a common, substrate-like

standard mechanism (Bode, W. and Huber, R., **Eur. J. Biochem. 204**:433-451 (1992). In particular, they all possess an exposed active site-binding loop with a characteristic canonical conformation. The binding loop on the A2AP model was therefore modelled on the equivalent loop of BPTI (residues 13 to 19), using atomic co-ordinates from the PDB entry 2PTC (in which BPTI is complexed with trypsin).

The complex of A2AP and the plasmin serine protease domain was modelled using the trypsin/BPTI complex structure from PDB entry 2PTC. The A2AP model was fitted to the BPTI structure by optimising the RMS difference between the co-ordinates of the backbone atoms in the active site-binding loops of the two inhibitors. The plasmin serine protease domain model was fitted to the trypsin structure by optimising the RMS difference between the co-ordinates of the C-alpha atoms of the conserved residues in an optimal sequence alignment of the two proteins. The A2AP/plasmin complex model was then refined by energy-minimisation.

The homology modelling was performed on a Silicon Graphics Indigo workstation using the Quanta molecular modelling program from Molecular Simulations Incorporated. Sequence alignments were produced using Quanta, the GCG sequence analysis software from the University of Wisconsin (Devereux, Haeberli and Smithies, **Nucleic Acids Research 12(1)**:387-395 (1984), and proprietary sequence alignment software. However, the actual method by which the homology models were built is not critical to this invention.

The trypsin and BPTI sequences used in the homology modelling were obtained from the Brookhaven Protein Data

Bank atomic co-ordinate entry 2PTC, the thrombin sequence was obtained from the PPACK/thrombin co-ordinate file, the plasminogen sequence from the SWISSPROT database entry PLMN_HUMAN, and the A2AP sequence from the SWISSPROT entry A2AP_HUMAN.

2. **Mung Bean Nuclease Digestion**

10 units of mung bean nuclease was added to approximately 1 µg DNA which had been digested with a restriction enzyme in a buffer containing 30mM NaOAc pH5.0, 100mM NaCl, 2mM ZnCl, 10% glycerol. The mung bean nuclease was incubated at 37° for 30 minutes, inactivated for 15 minutes at 67° before being phenol extracted and ethanol precipitated.

3. **Oligonucleotide synthesis**

The oligonucleotides were synthesised by automated phosphoramidite chemistry using cyanoethyl phosphoramidites. The methodology is now widely used and has been described (Beaucage, S.L. and Caruthers, M.H. Tetrahedron Letters 24, 245 (1981) and Caruthers, M.H. Science 230, 281-285 (1985)).

4. **Purification of Oligonucleotides**

The oligonucleotides were de-protected and removed from the CPG support by incubation in concentrated NH3. Typically, 50 mg of CPG carrying 1 micromole of oligonucleotide was de-protected by incubation for 5 hours at 70° in 600 µl of concentrated NH3. The supernatant was transferred to a fresh tube and the oligomer precipitated with 3 volumes of ethanol. Following centrifugation the pellet was dried and

resuspended in 1 ml of water. The concentration of crude oligomer was then determined by measuring the absorbance at 260 nm. For gel purification 10 absorbance units of the crude oligonucleotide was dried down and resuspended in 15 µl of marker dye (90% de-ionised formamide, 10mM tris, 10 mM borate, 1mM EDTA, 0.1% bromophenol blue). The samples were heated at 90° for 1 minute and then loaded onto a 1.2 mm thick denaturing polyacrylamide gel with 1.6 mm wide slots. The gel was prepared from a stock of 15% acrylamide, 0.6% bisacrylamide and 7M urea in 1 X TBE and was polymerised with 0.1% ammonium persulphate and 0.025% TEMED. The gel was pre-run for 1 hr. The samples were run at 1500 V for 4-5 hours. The bands were visualised by UV shadowing and those corresponding to the full length product cut out and transferred to micro-testubes. The oligomers were eluted from the gel slice by soaking in AGEB (0.5 M ammonium acetate, 0.01 M magnesium acetate and 0.1% SDS) overnight. The AGEB buffer was then transferred to fresh tubes and the oligomer precipitated with three volumes of ethanol at 70° for 15 mins. The precipitate was collected by centrifugion in an Eppendorf microfuge for 10 mins, the pellet washed in 80% ethanol, the purified oligomer dried, redissolved in 1 ml of water and finally filtered through a 0.45 micron micro-filter. (The word EPPENDORF is a trade mark.) The concentration of purified product was measured by determining its absorbance at 260 nm.

5. **Kinasing of Oligomers**

100 pmole of oligomer was dried down and resuspended in 20 µl kinase buffer (70 mM Tris pH 7.6, 10 mM MgCl2, 1 mM ATP, 0.2mM spermidine, 0.5 mM dithiothreitol). 10 u of T4 polynucleotide kinase was added and the mixture incubated at 37° for 30 mins. The kinase was then inactivated by

heating at 70° for 10 mins.

6. **Dideoxy Sequencing**

The protocol used was essentially as has been described (Biggin, M.D., Gibson, T.J., Hong, G.F. P.N.A.S. 80 3963-3965 (1983). Where appropriate the method was modified to allow sequencing on plasmid DNA as has been described (Guo, L-H., Wu R Nucleic Acids Research 11 5521-5540 (1983).

7. **Transformation**

Transformation was accomplished using standard procedures. The strain used as a recipient in the cloning using plasmid vectors was HW87 or DH5 which has the following genotype:

araD139(ara-leu)del7697 (lacIPOZY)del74 galU galK hsdR rpsL srl recA56

RZ1032 is a derivative of E. coli that lacks two enzymes of DNA metabolism: (a) dUTPase (dut) which results in a high concentration of intracellular dUTP, and (b) uracil N-glycosylase (ung) which is responsible for removing mis incorporated uracils from DNA (Kunkel et al, Methods in Enzymol., 154, 367-382 (1987)). Its principal benefit is that these mutations lead to a higher frequency of mutants in site directed mutagenesis. RZ1032 has the following genotype:

HfrKL16PO/45[lysA961-62), dut1, ung1, thi1, re[A], Zbd-279::Tn10, supE44

JM103 is a standard recipient strain for manipulations involving M13 based vectors.

8. **Site Directed Mutagenesis**

Kinased mutagenesis primer (2.5pmole) was annealed to the single stranded template DNA, which was prepared using RZ1032 as host, (1 µg) in a final reaction mix of 10 µl containing 70 mM Tris, 10 mM MgCl2. The reaction mixture in a polypropylene micro-testube (EPPENDORF) was placed in a beaker containing 250 ml of water at 70°C for 3 minutes followed by 37°C for 30 minutes. The annealed mixture was then placed on ice and the following reagents added: 1 µl of 10 X TM (700 mM Tris, 100 mM MgCl2 pH7.6), 1 µl of a mixture of all 4 deoxyribonucleotide triphosphates each at 5mM, 2 µl of T4 DNA ligase (100u), 0.5 µl Klenow fragment of DNA polymerase and 4.5 µl of water. The polymerase reaction mixture was then incubated at 15° for 4-16 hrs. After the reaction was complete, 180 µl of TE (10 mM Tris, 1 mM EDTA pH8.0) was added and the mutagenesis mixture stored at -20°C.

For the isolation of mutant clones the mixture was then transformed into the recipient JM103 as follows. A 5 ml overnight culture of JM103 in 2 X YT (1.6% Bactotryptone, 1% Yeast Extract, 1% NaCl) was diluted 1 in a 100 into 50 ml of pre-warmed 2 X YT. The culture was grown at 37° with aeration until the A600 reached 0.4. The cells were pelleted and resuspended in 0.5 vol of 50 mM CaCl2 and kept on ice for 15 mins. The cells were then re-pelleted at 4° and resuspended in 2.5 ml cold 50 mM CaCl2. For the transfection, 0.25, 1, 2, 5, 20 and 50 µl aliquots of the mutagenesis mixture were added to 200 µl of competent cells which were kept on ice for 30 mins. The cells were then heated shocked at 42° for 2 mins. To each tube was then added 3.5 ml of YT soft agar containing 0.2 ml of a

late exponential culture of JM103, the contents were mixed briefly and then poured onto the surface of a pre-warmed plate containing 2 X YT solidified with 1.5% agar. The soft agar layer was allowed to set and the plates then incubated at 37° overnight.

Single stranded DNA was then prepared from isolated clone as follows: Single plaques were picked into 4 ml of 2 X YT that had been seeded with 10 µl of a fresh overnight culture of JM103 in 2 X YT. The culture was shaken vigorously for 6 hrs. 0.5ml of the culture was then removed and added to 0.5 ml of 50% glycerol to give a reference stock that was stored at -20°. The remaining culture was centrifuged to remove the cells and 1 ml of supernatant carrying the phage particles was transferred to a fresh EPPENDORF tube. 250 µl of 20% PEG6000, 250mM NaCl was then added, mixed and the tubes incubated on ice for 15 mins. The phage were then pelleted at 10,000 rpm for 10 mins, the supernatant discarded and the tubes re-centrifuged to collect the final traces of PEG solution which could then be removed and discarded. The phage pellet was thoroughly resuspended in 200 µl of TEN (10 mM Tris, 1 mM EDTA, 0.3 M NaOAc). The DNA was isolated by extraction with an equal volume of Tris saturated phenol. The phases were separated by a brief centrifugation and the aqueous phase transferred to a clean tube. The DNA was re-extracted with a mixture of 100 µl of phenol, 100 µl chloroform and the phases again separated by centrifugation. Traces of phenol were removed by three subsequent extractions with chloroform and the DNA finally isolated by precipitation with 2.5 volumes of ethanol at -20° overnight. The DNA was pelleted at 10,000 rpm for 10 min, washed in 70% ethanol, dried and finally resuspended in 50 µl of TE.

9. Electroporation

Chinese hamster ovary cells (CHO) or the mouse myeloma cell line p3x63-Ag8.653 were grown and harvested in mid log growth phase. The cells were washed and resuspended in PBS and a viable cell count was made. The cells were then pelleted and resuspended at 1 X 107 cells/ml. 40 µg of linearised DNA was added to 1 ml of cells and allowed to stand on ice for 15 mins. One pulse of 800 V/ 25 µF was administered to the cells using a commercially available electroporation apparatus (BIORAD GENE PULSER - trade mark). The cells were incubated on ice for a further 15 mins and then plated into 5 X 96 well plates with 200 µl of medium per well (DMEM, 5% FCS, Pen/Strep, glutamine) or 3 x 9cm dishes with 10 mls medium in each dish and incubated overnight. After 24 hrs the medium was removed and replaced with selective media containing xanthine (250µg/ml), mycophenolic acid (5µg/ml) and 1 x hypoxanthine-thymidine (HT). The cells were fed every third day. After about 14 days gpt resistant colonies are evident in some of the wells and on the plates. The plates were screened for plasminogen by removing an aliquot of medium from each well or plate and assayed using an ELISA assay. Clones producing plasminogen were scaled up and the expression level monitored to allow the selection of the best producer.

10. ELISA for Human Plasminogen

ELISA plates (Pro-Bind, Falcon) are coated with 50 µl/well of goat anti-human plasminogen serum (Sigma) diluted 1:1000 in coating buffer (4.0g $Na_2CO_3(10.H_2O)$, 2.93g $NaHCO_3$ per litre H_2O, pH 9.6) and incubated overnight at 4°C. Coating solution is then removed and plates are blocked by incubating with 50 µl/well of

PBS/0.1% casein at room temperature for 15 minutes. Plates are then washed 3 times with PBS/0.05% Tween 20. Samples of plasminogen or standards diluted in PBS/Tween are added to the plate and incubated at room temperature for 2 hours. The plates are then washed 3 times with PBS/Tween and then 50 µl/well of a 1:1000 dilution in PBS/Tween of a monoclonal antihuman plasminogen antibody (eg #3641 and #3642 from American Diagnostica, New York, USA) is added and incubated at room temperature for 1 hour. The plates are again washed 3 times with PBS/Tween and then 50 µl/well of horse radish peroxidase conjugated goat anti-mouse IgG (Sigma) is added and incubated at room temperature for 1 hour. Alternatively, the bound plasminogen is revealed by incubation with 50µl/well of horse radish peroxidase conjugated sheep anti-human plasminogen (The Binding Site). The plates are washed 5 times with PBS/Tween and then incubated with 100 µl/well of peroxidase substrate (0.1M sodium acetate/ citric acid buffer pH 6.0 containing 100mg/litre 3,3',5,5'-tetramethyl benzidine and 13mM H_2O_2. The reaction is stopped after approximately 5 minutes by the addition of 25 µl/well of 2.5M sulphuric acid and the absorbance at 450nm read on a platereader.

11. **Purification of Plasminogen Variants**

Plasminogen variants are purified in a single step by chromatography on lysine SEPHAROSE 4B (Pharmacia). A column is equilibrated with at least 10 column volumes of 0.05M sodium phosphate buffer pH 7.5. The column is loaded with conditioned medium at a ratio of 1ml resin per 0.6mg of plasminogen variant as determined by ELISA using human glu-plasminogen as standard. Typically 400 ml of conditioned medium containing plasminogen are applied to a 10 ml column (H:D=4) at a linear flow rate of 56

ml/cm/h at 4°C. After loading is complete, the column is washed with a minimum of 5 column volumes of 0.05M phosphate buffer pH 7.5 containing 0.5M NaCl until non-specifically bound protein ceases to be eluted. Desorption of bound plasminogen is achieved by the application of 0.2M epsilon-amino-caproic acid in de-ionised water pH 7.0. Elution requires 2 column volumes and is carried out at a linear flow rate of 17ml/cm/h. Following analysis by SDS PAGE to check purity, epsilon-amino-caproic acid is subsequently removed and replaced with a suitable buffer, eg Tris, PBS, HEPES or acetate, by chromatography on pre-packed, disposable, PD10 columns containing SEPHADEX G-25M (Pharmacia). (The word SEPHADEX is a trade mark.) Typically, 2.5ml of each plasminogen mutant at a concentration of 0.3mg/ml are processed in accordance with the manufacturers' instructions. Fractions containing plasminogen, as determined by A280 are then pooled.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: British Bio-technology Limited
(B) STREET: Watlington Road
(C) CITY: Cowley, Oxford
(E) COUNTRY: GB
(F) POSTAL CODE (ZIP): OX4 5LY

(ii) TITLE OF INVENTION: MODIFIED PROTEASES

(iii) NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:
Not Applicable

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 690 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..690
(D) OTHER INFORMATION: /partial
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/function= "encodes plasmin protease domain"
/product= "nucleotide with corresponding protein"
/number= 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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  288
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  336
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            100                 105                 110

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  384
Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly Trp Gly Glu Thr Gln
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  432
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  576
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 230 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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35 40 45

Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His Gln Glu Val Asn
50 55 60

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Pro Thr Arg Lys Asp Ile Ala Leu Leu Lys Leu Ser Ser Pro Ala Val
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Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser Pro Asn Tyr Val
100 105 110

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(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /function= "MUTAGENESIS PRIMER FOR A1"
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(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
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/product= "SYNTHETIC DNA"

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(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24
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/product= "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /function= "MUTAGENESIS PRIMER FOR A12"
/product= "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /function= "MUTAGENESIS PRIMER FOR A14"
/product= "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

CTTGGGGACT TGGCTAGACA GTGG
24

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..25
(D) OTHER INFORMATION: /function= "MUTAGENESIS PRIMER FOR A15"
/product= "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTTGGGGACT TCCTTAGACA GTGGG
25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE: synthetic DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(C) OTHER INFORMATION: /function= "MUTAGENESIS PRIMER FOR A16"
/product= "SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTTCGAGATT CACTGCTTGG TGTGCAC
27

Figure 1 - Human Serine Proteases

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                          1                                                  50
Complement Factor B  WEHRKGTDYH KQPWQAKISV IRPSKGH..E SCMGAVVSEY FVLTAAHCF.
     Complement C2   GVGNMSANAS DQERTPWHVT IKP.KSQ..E TCRGALISDQ WVLTAAHCF.
       Medullasin    IVGGRRARPH AWPFMVSLQL R...GG...H FCGATLIAPN FVMSAAHCV.
      Myeloblastin   .......... ....MASLQM RGNPGS...H FCGGTLIHPS FVLTAAHCL.
     Complement C1S  IIGGSDADIK NFPWQVFF.. ..D.NP.... WAGGALINEY WVLTAAHVV.
     Complement C1R  IIGGQKAKMG NFPWQVFT.. ..NIHG.... RGGGALLGDR WILTAAHTL.
          Factor X   IVGGQECKDG ECPWQALLI. NEENEG.... FCGGTILSEF YILTAAHCL.
         Factor IX   VVGGEDAKPG QFPWQVVL.. NGKVDA.... FCGGSIVNEK WIVTAAHCV.
        Factor VII   IVGGKVCPKG ECPWQVLLL. VNGAQ..... LCGGTLINTI WVVSAAHCF.
         Protein C   LIDGKMTRRG DSPWQVVLL. DSKKKL.... ACGAVLIHPS WVLTAAHCM.
          Thrombin   IVEGSDAEIG MSPWQVMLFR KSPQEL.... LCGASLISDR WVLTAAHCLL
             u-PA    IIGGEFTTIE NQPWFAAIYR RH.RGGSVTY VCGGSLMSPC WVISATHCF.
             t-PA    IKGGLFADIA SHPWQAAIFA KHRRSPGERF LCGGILISSC WILSAAHCF.
        Factor XII   VVGGLVALRG AHPYIAALYW GHS....... FCAGSLIAPC WVLTAAHCL.
  Apolipoprotein A   IVGGCVAHPH SWPWQVSL.R .TRFGK...H FCGGTLISPE WVLTAAHCL.
           Plasmin   VVGGCVAHPH SWPWQVSL.R .TRFGM...H FCGGTLISPE WVLTAAHCL.
             Hepsin  IVGGRDTSLG RWPWQVSL.R .YD.GA...H LCGGSLLSGD WVLTAAHCF.
       Elastase IIIa VVHGEDAVPY SWPWQVSL.Q .YEKSGSFYH TCGGSLIAPD WVVTAGHCI.
       Elastase IIIb VVNGEDAVPY SWPWQVSL.Q .YEKSGSFYH TCGGSLIAPD WVVTAGHCI.
        Elastase IIa VVGGEEARPN SWPWQVSL.Q .YSSNGKWYH TCGGSLIANS WVLTAAHCI.
        Elastase IIb MLGGEEARPN SWPWQVSL.Q .YSSNGQWYH TCGGSLIANS WVLTAAHCI.
     Chymotrypsin B  IVNGEDAVPG SWPWQVSL.Q .DKTG...FH FCGGSLISED WVVTAAHCG.
      Alpha Tryptase IVGGQEAPRS KWPWQVSL.R .VR.DRYWMH FCGGSLIHPQ WVLTAAHCL.
       Beta Tryptase IVGGQEAPRS KWPWQVSL.R .VH.GPYWMH FCGGSLIHPQ WVLTAAHCV.
          Factor XI  IVGGTASVRG EWPWQVTL.H .TT.SPTQRH LCGGSIIGNQ WILTAAHCF.
   Plasma Kallikrein IVGGTNSSWG EWPWQVSL.Q .VK.LTAQRH LCGGSLIGHQ WVLTAAHCF.
             Acrosin IVGGKAAQHG AWPWMVSL.Q IFRYNSHRYH TCGGSLLNSR WVLTAAHCF.
           Trypsin I IVGGYNCEEN SVPYQVSL.. ..NS.G..YH FCGGSLINEQ WVVSAGHCY.
          Trypsin II IVGGYICEEN SVPYQVSL.. ..NS.G..YH FCGGSLISEQ WVVSAGHCY.
         Trypsin III IVGGYTCEEN SLPYQVSL.. ..NS.G..SH FCGGSLISEQ WVVSAAHCY.
Tissue Kallikrein 2  IVGGWECEKH SQPWQVAV.. ..YSHG..WA HCGGVLVHPQ WVLTAAHCL.
             PSA     IVGGWECEKH SQPWQVLV.. ..ASRG..RA VCGGVLVHPQ WVLTAAHCI.
Tissue Kallikrein 1  IVGGWECEQH SQPWQAAL.. ..YHFS..TF QCGGILVHRQ WVLTAAHCI.
        Granzyme B   IIGGHEAKPH SRPYMAYL.M IWDQKS..LK RCGGFLIQDD FVLTAAHCW.
     T-cell Granzyme IIGGHEAKPH SRPYMAFV.Q FLQEKS..RK RCGGILVRKD FVLTAAHCQ.
        Cathepsin G  IIGGRESRPH SRPYMAYL.Q IQSPAG..QS RCGGFLVRED FVLTAAHCW.
  Complement Factor D ILGGREAEAH ARPYMASV.Q L...NG..AH LCGGVLVAEQ WVLSAAHCL.
        Granzyme A   IIGGNEVTPH SRPYMVLL.S L...DR..KT ICAGALIAKD WVLTAAHC..
  Complement Factor I IVGGKRAQLG DLPWQVAIKD ASGIT..... .CGGIYIGGC WILTAAHCL.
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                    51                                                  100
Complement Factor B ...TVDDKEH SI.KVSVGGE K....RDLEI EVVLFHPNYN INGKKEAGIP
     Complement C2  ...R.DGNDH SLWRVNVGDP KSQWGKELLI EKAVISPGFD VFAKKNQGIL
       Medullasin   .....ANVNV RAVRVVLGAH NLSRREPTRQ VFAVQRIFEN GYDPVNLL..
     Myeloblastin   .....RDIPQ RLVNVVLGAH NVRTQEPTQQ HFSVAQVFLN NYDAENKL..
     Complement C1S .......EGN REPTMYVGST SVQTSRLAKS KMLTPEHVFI HPGWKLLEVP
Complement C1R      YPKEHEAQSN ASLDVFLGHT NVEE..LMKL GNHPIRRVSV HPDYR....Q
          Factor X  ....YQAK.. .RFKVRVGDR NTEQEEGG.E AVHEVEVVIK HNRF......
         Factor IX  ....ETGV.. .KITVVAGEH NIEETEHT.E QKRNVIRIIP HHNYNA....
        Factor VII  ....DKIKNW RNLIAVLGEH DLSEHDGD.E QSRRVAQVII PSTYVP....
         Protein C  ....DESK.. .KLLVRLGEY DLRRWEKW.E LDLDIKEVFV HPNY......
          Thrombin  YPPWDKNFTE NDLLVRIGKH SRTRYERNIE KISMLEKIYI HPRYNW....
             u-PA   .IDYPKKE.. .DYIVYLGRS RLNSNTQGEM KF........ ..EVENLILH
             t-PA   .QERFPPH.. .HLTVILGRT YRVVPGEEEQ KF........ ..EVEKYIVH
        Factor XII  .QDRPAPE.. .DLTVVLGQE RRNHSCEPCQ TL........ ..AVRSYRLH
Apolipoprotein A    .K..KSSRP. SSYKVILGAH QEV...NLES HV.....QE. ..IEVSRLFL
           Plasmin  .E..KSPRP. SSYKVILGAH QEV...NLEP HV.....QE. ..IEVSRLFL
            Hepsin  .P..ERNRVL SRWRVFAGAV AQASPHGLQL GV.....QA. ..VVYHGGYL
     Elastase IIIa  .S..RD.... LTYQVVLGEY NLAVKEGPEQ VI.....PI. ..NSEELFVH
     Elastase IIIb  .S..SS.... RTYQVVLGEY DRAVKEGPEQ VI.....PI. ..NSGDLFVH
      Elastase IIa  .S..SS.... RTYRVGLGRH NLYVAESGSL AV.....SV. ....SKIVVH
      Elastase IIb  .S..SS.... RIYRVMLGQH NLYVAESGSL AV.....SV. ....SKIVVH
  Chymotrypsin B    .V..RT.... SDV.VVAGEF DQGSDEENIQ VL.....KI. ....AKVFKN
    Alpha Tryptase  .G..PDVKDL ATLRVN.SGT HLYYQDQLLP VS.....RI. ..MVHPQFYI
     Beta Tryptase  .G..PDVKDL AALRVQLREQ HLYYQDQLLP VS.....RI. ..IVHPQFYT
          Factor XI .YGVESPKIL RVYSGILNQS EIKEDTSFFG VQ.....EI. ..IIHDQYKM
  Plasma Kallikrein .DGLPLQDVW RIYSGILNLS DITKDTPFSQ IK.....EI. ..IIHQNYKV
            Acrosin .VGKNNVHD. ..WRLVFGAK EITYGNNKPV KA.....PLQ ERYVEKIIIH
          Trypsin I  .....KSRI. ...QVRLGEH NIEVLEGNEQ F.INAAKIIR HPQYDRKTLN
         Trypsin II  .....KSRI. ...QVRLGEH NIEVLEGNEQ F.INAAKIIR HPKYNSRTLD
        Trypsin III  .....KTRI. ...QVRLGEH NIKVLEGNEQ F.INAAKIIR HPKYNRDTLD
Tissue Kallikrein 2  .....KKNS. ...QVWLGRH NLFEPEDTGQ R.VPVSHSFP HPLYNMSLLK
                PSA  .....RNKS. ...VILLGRH SLFHPEDTGQ V.FQVSHSFP HPLYDMSLLK
Tissue Kallikrein 1  .....SDNY. ...QLWLGRH NLFDDENTAQ F.VHVSESFP HPGFNMSLLE
          Granzyme B .....GSSIN ....VTLGAH NIKEQEPTQQ F.IPVKRPIP HPAYNPKNFS
      T-cell Granzyme .....GSSIN ....VTLGAH NIKEQERTQQ F.IPVKRPIP HPAYNPKNFS
         Cathepsin G  .....GSNIN ....VTLGAH NIQRRENTQQ H.ITARRAIR HPQYNQRTIQ
Complement Factor D   .....EDAAD GKVQVLLGAT HLPQPEPXXX ITIEVLRAVP HPDSQPDTID
          Granzyme A  .......NLN KRSQVILGAH SITREEPTKQ IML.VKKEFP YPCYDPATRE
Complement Factor I   .....RASKT HRYQIWTTVV DWIHPDLKRI VIEYVDRIIF HENYNA....
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	101			150
Complement Factor B	EFY.....	DYDVALIKL.	....KNKLKY	GQTIRPICLP CTEGTTRALR
Complement C2	EFY.....	GDDIALLKL.	....AQKVKM	STHARPICLP CTMEANLALR
Medullasin	.....	.NDIVILQL.	....NGSATI	NANVQVAQLP AQGR....RL
Myeloblastin	.....	.NDILLIQL.	....SSPANL	SASVTSVQLP QQDQ....PV
Complement C1S	E....GRTNF	DNDIALVRL.	....KDPVKM	GPTVSPICLP GTSSDYNLMD
Complement C1R	D....ESYNF	EGDIALLEL.	....ENSVTL	GPNLLPICLP DNDTFYDL..
Factor X	.....TKETY	DFDIAVLRL.	....KTPITF	RMNVAPACLP ERDWAESTL.
Factor IX	.....AINKY	NHDIALLEL.	....DEPLVL	NSYVTPICIA DKEYTN.IF.
Factor VII	.....G..TT	NHDIALLRL.	....HQPVVL	TDHVVPLCLP ERTFSERTL.
Protein C	.....SKSTT	DNDIALLHL.	....AQPATL	SQTIVPICLP DSGLAERELN
Thrombin	......RENL	DRDIALMKL.	....KKPVAF	SDYIHPVCLP DRETAASLLQ
u-PA	KDYSADTLAH	HNDIALLKIR	SK.EGRCAQP	SRTIQTICLP SMY..NDPQF
t-PA	KEFDDDT..Y	DNDIALLQLK	SD.SSRCAQE	SSVVRTVCLP P....ADLQL
Factor XII	EAFS..PVSY	QHDLALLRLQ	EDADGSCALL	SPYVQPVCLP SGA..ARP..
Apolipoprotein A	.......EPT	QADIALLKL.	...SRPAV.I	TDKVMPACLP SPD..YMVT.
Plasmin	.......EPT	RKDIALLKL.	...SSPAV.I	TDKVIPACLP SPN..YVVA.
Hepsin	PFRDPNSEEN	SNDIALVHL.	...SSPLP.L	TEYIQPVCLP AAG..QALV.
Elastase IIIa	PLWNRSCVAC	GNDIALIKL.	...SRSAQ.L	GDAVQLASLP PAG..DILP.
Elastase IIIb	PLWNRSCVAC	GNDIALIKL.	...SRSAQ.L	GDAVQLASLP PAG..DILP.
Elastase IIa	KDWNSNQISK	GNDIALLKL.	...ANPVS.L	TDKIQLACLP PAG..TILP.
Elastase IIb	KDWNSNQVSK	GNDIALLKL.	...ANPVS.L	TDKIQLACLP PAG..TILP.
Chymotrypsin B	PKF..SILTV	NNDITLLKL.	...ATPAR.F	SQTVSAVCLP SAD..DDFP.
Alpha Tryptase	.......IQT	GADIALLEL.	...EEPVN.I	SSRVHTVMLP PAS..ETFP.
Beta Tryptase	.......AQI	GADIALLEL.	...EEPVK.V	SSHVHTVTLP PAS..ETFP.
Factor XI	.......AES	GYDIALLKL.	...ETTVN.Y	TDSQRPICLP SKG..DRNV.
Plasma Kallikrein	.......SEG	NHDIALIKL.	...QAPLN.Y	TEFQKPICLP SKG..DTST.
Acrosin	EKYNS..ATE	GNDIALVEI.	...TPPIS.C	GRFIGPGCLP HFK..AGLP.
Trypsin I	N.....	..DIMLIKL.	...SSRA.VI	NARVSTISLP TAP..PAT..
Trypsin II	N.....	..DILLIKL.	...SSPA.VI	NSRVSAISLP TAP..PAA..
Trypsin III	N.....	..DIMLIKL.	...SSPA.VI	NARVSTISLP TAP..PAA..
Tissue Kallikrein 2	HQSLRPDEDS	SHDLMLLRL.	...SEPAK.I	TDVVKVLGLP TQE..PAL..
PSA	NRFLRPGDDS	SHDLMLLRL.	...SEPAE.L	TDAVKVMDLP TQE..PAL..
Tissue Kallikrein 1	NHTRQADEDY	SHDLMLLRL.	...TEPADTI	TDAVKVVELP TQE..PEV..
Granzyme B	N.....	..DIMLLQL.	...ERKAK.R	TRAVQPLRLP SNK..AQVK.
T-cell Granzyme	N.....	..DIMLLQL.	...ERKAK.W	TTAVRPLRLP SSK..AQVK.
Cathepsin G	N.....	..DIMLLQL.	...SRRVR.R	NRNVNPVALP RAQ..EGLR.
Complement Factor D	H.....	..DLLLLQL.	...SEKAT.L	GPAVRPLPWQ RVD..RDVA.
Granzyme A	G.....	..DLKLLQL.	...TEKAK.I	NKYVTILHLP KKG..DDVK.
Complement Factor I	.....GTY	QNDIALIEMK	KDGNKKDCEL	.....PRSIP ACVPWSPYLF

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                    151                                                 200
Complement Factor B LPPTTTCQQQ KEELLPAQDI KALFVSEEEK KLTRKEVYIK NGDKKGSC.E
     Complement C2  RPQGSTCRDH ENELLNKQSV PAHFVALNGS KL...NINLK MGVEWTSCAE
       Medullasin   GNGVQCLAMG WGLL..... GRNRGIASVL QELNVTV... ..VT......
     Myeloblastin   PHGTQCLAMG WGRV..... GAHDPPAQVL QELNVTV... ..VT......
     Complement C1S GDL..GLISG WGRTEK.... ...RDRAVRL KAARLPV... ..APLRKCKE
     Complement C1R GLM..GYVSG FGVMEE.... ...KI.AHDL RFVRLPV... ..ANPQACEN
           Factor X MTQKTGIVSG FGRTHE.KGR QS.....TRL KMLEVPY... ..VDRNSCKL
          Factor IX LKFGSGYVSG WGRVFH.KGR SA.....LVL QYLRVPL... ..VDRATCLR
         Factor VII AFVRFSLVSG WGQLLD.RGA TA.....LEL MVLNVPR... ..LMTQDCLQ
          Protein C QAGQETLVTG WGYHSS.REK EAKRNRTFVL NFIKIPV... ..VPHNECSE
          Thrombin  AGYK.GRVTG WGNLKETWTA NVGKGQPSVL QVVNLPI... ..VERPVCKD
              u-PA  G..TSCEITG FGKENS.... TDYLYPEQ.L KMTVVKL... ..ISHRECQQ
              t-PA  PDWTECELSG YGKHEA.... LSPFYSER.L KEAHVRL... ..YPSSRCTS
         Factor XII SETTLCQVAG WGHQFE.... GAEEYASF.L QEAQVPF... ..LSLERCSA
Apolipoprotein A    ARTE.CYITG WGETQG.... TFG..TG.LL KEAQLLV... ..IENEVCNH
            Plasmin DRTE.CFITG WGETQG.... TFG..AG.LL KEAQLPV... ..IENKVCNR
              Hepsin DGKI.CTVTG WGNTQ..... YYGQQAG.VL QEARVPI... ..ISNDVCNG
       Elastase IIIa NKTP.CYITG WGRLYT.... NGP.LPD.KL QQARLPV... ..VDYKHCSR
       Elastase IIIb NETP.CYITG WGRLYT.... NGP.LPD.KL QEALLPV... ..VDYEHCSR
        Elastase IIa NNYP.CYVTG WGRLQT.... NGA.VPD.VL QQGRLLV... ..VDYATCSS
        Elastase IIb NNYP.CYVTG WGRLQT.... NGA.LPD.DL KQGRLLV... ..VDYATCSS
     Chymotrypsin B AGTL.CATTG WGKTKY.... NANKTPD.KL QQAALPL... ..LSNAECKK
       Alpha Tryptase PGMP.CWVTG WGDVDN.... DEPLPPPFPL KQVKVPI... ..MENHICDA
        Beta Tryptase PGMP.CWVTG WGDVDN.... DERLPPPFPL KQVKVPI... ..MENHICDA
            Factor XI  IYTD.CWVTG WGYRKL.... RDKIQN..TL QKAKIPL... ..VTNEECQK
  Plasma Kallikrein    IYTN.CWVTG WGFSKE.... KGEIQN..IL QKVNIPL... ..VTNEECQK
             Acrosin   RGSQSCWVAG WGYIEE.... KAP.RPSSIL MEARVDL... ..IDLDLCNS
           Trypsin I   .GTK.CLISG WGNTAS.... SGADYPD.EL QCLDAPV... ..LSQAKCEA
          Trypsin II   .GTE.SLISG WGNTLS.... SGADYPD.EL QCLDAPV... ..LSQAECEA
         Trypsin III   .GTE.CLISG WGNTLS.... FGADYPD.EL KCLDAPV... ..LREAECKA
Tissue Kallikrein 2    .GTT.CYASG WGSIEP.... EEFLRPR.SL QCVSLHL... ..LSNDMCAR
                PSA    .GTT.CYASG WGSIEP.... EEFLTPK.KL QCVDLHV... ..ISNDVCAQ
Tissue Kallikrein 1    .GST.CLASG WGSIEP.... ENFSFPD.DL QCVDLKI... ..LPNDECEK
         Granzyme B    PGQT.CSVAG WGQTAP.... LG.KHSH.TL QEVKMTV... ..QEDRKCES
      T-cell Granzyme  PGQL.CSVAG WG.YVS.... MS.TLAT.TL QEVLLTV... ..QKDCQCER
          Cathepsin G  PGTL.CTVAG WGR.VS.... MR.RGTD.TL REVQLRV... ..QRDRQCLR
Complement Factor D    PGTL.CDVAG WGIVNH.... AG.RRPD.SL QHVLLPV... ..LDRATCRL
         Granzyme A    PGTM.CQVAG WGRTHN.... SA.SWSD.TL REVNITI... ..IDRKVCND
Complement Factor I    QPNDTCIVSG WGREKDNERV FSLQWGEVKL ISNCSKF... ..YGNRFYEK
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	201				250
Complement Factor B	RDAQYAPGYD	KVKDISEVVT	PRFLCTGGVS	PYADPNTCRG	DSGGPLIVHK
Complement C2	VVSQEKTMFP	NLTDVREVVT	DQFLCSGTQ.	..EDESPCKG	ESGGAVFLER
Medullasin	....	........SL	CRRSNVCTLV	RGRQAGVCFG	DSGSPLVCNG
Myeloblastin	....	........FF	CRPHNICTFV	PRRKAGICFG	DSGGPLICDG
Complement C1S	VKVEKPTADA	EAYVFTPNMI	CAG....GEK	G...MDSCKG	DSGGAFAVQD
Complement C1R	WLRGKNRMD.	...VFSQNMF	CAGH...PSL	K...QDACQG	DSGGVFAVRD
Factor X	....SSSFI.	....ITQNMF	CAGY...DTK	Q...EDACQG	DSGGPHV..T
Factor IX	....STKFT.	....IYNNMF	CAGF...HEG	G...RDSCQG	DSGGPHV..T
Factor VII	....QSRKVG	DSPNITEYMF	CAGY...SDG	S...KDSCKG	DSGGPHA..T
Protein C	....VMSNM.	....VSENML	CAGI...LGD	R...QDACEG	DSGGPMV..A
Thrombin	....STRI..	...RITDNMF	CAGYKPDEGK	R...GDACEG	DSGGPFVMKS
u-PA	PHYYGS....	...EVTTKML	CAADPQWKT.	.....DSCQG	DSGGPLVCSL
t-PA	QHLLNR....	...TVTDNML	CAGDTRSGGP	QANLHDACQG	DSGGPLVCLN
Factor XII	PDVHGS....	...SILPGML	CAGFLEGGT.	.....DACQG	DSGGPLVCED
Apolipoprotein A	YKY.....	I	CAEHLARGT.	.....DSCQG	DSGGPLVCFE
Plasmin	YEFLNG....	...RVQSTEL	CAGHLAGGT.	.....DSCQG	DSGGPLVCFE
Hepsin	ADFYGN....	...QIKPKMF	CAGYPEGGI.	.....DACQG	DSGGPFVCED
Elastase IIIa	WNWWGS....	...TVKKTMV	CAG.GY.IR.	.....SGCNG	DSGGPLNCPT
Elastase IIIb	WNWWGS....	...SVKKTMV	CAG.GD.IR.	.....SGCNG	DSGGPLNCPT
Elastase IIa	SAWWGS....	...SVKTSMI	CAG.GDGVI.	.....SSCNG	DSGGPLNCQA
Elastase IIb	SGWWGS....	...TVKTNMI	CAG.GDGVI.	.....CTCNG	DSGGPLNCQA
Chymotrypsin B	S..WGR....	...RITDVMI	CAG.ASGV..	.....SSCMG	DSGGPLVCQ.
Alpha Tryptase	KYHLGAYTGD	DVRIIRDDML	CAG..NSQR.	.....DSCKG	DSGGPLVCKV
Beta Tryptase	KYHLGAYTGD	DVRIVRDDML	CAG..NTRR.	.....DSCQG	DSGGPLVCKV
Factor XI	RYR.....	.GHKITHKMI	CAGYREGGK.	.....DACKG	DSGGPLSCKH
Plasma Kallikrein	RYQ.....	.DYKITQRMV	CAGYKEGGK.	.....DACKG	DSGGPLVCKH
Acrosin	TQWYNG....	...RVQPTNV	CAGYPVGKI.	.....DTCQG	DSGGPLMCKD
Trypsin I	....S.....	YPGKITSNMF	CVGFLEGGK.	.....DSCQG	DSGGPVVCNG
Trypsin II	....S.....	YPGKITNNMF	CVGFLEGGK.	.....DSCQG	DSGGPVVSNG
Trypsin III	....S.....	CPGKITNSMF	CVGFLEGGK.	.....DSWKR	DSGGPVVCNG
Tissue Kallikrein 2	....A.....	YSEKVTEFML	CAGLWTGGK.	.....DTCGG	DSGGPLVCNG
PSA	....V.....	HPQKVTKFML	CAGRWTGGK.	.....STCSG	DSGGPLVCNG
Tissue Kallikrein 1	....A.....	HVQKVTDFML	CVGHLEGGK.	.....DTCVG	DSGGPLMCDG
Granzyme B	DLRHY.....	YDSTIEL...	CVGDPEIKK.	.....TSFKG	DSGGPLVCNK
T-cell Granzyme	LFHGN.....	YSRATEI...	CVGDPKKTQ.	.....TGFKG	DSGGPLVCKD
Cathepsin G	IF.GS.....	YDPRRQI...	CVGDRRERK.	.....AAFKG	DSGGPLLCNN
Complement Factor D	YD.....	.....VLRLM	CAESNR..R.	.....DSCKG	DSGGPLVCGG
Granzyme A	RNHYN.....	FNPVIGMNMV	CAGSLRGGR.	.....DSCNG	DSGSPLLCEG
Complement Factor I	....	.......EME	CAGTYDGSI.	.....DACKG	DSGGPLVCMD

	251				300
Complement Factor B	RS....RFIQ	VGVISWGVVD	VC...KNQKR	QKQVP....A	HARDFHINLF
Complement C2	RF....RFFQ	VGLVSWGLYN	PCLGSADKNS	RKRAPRSKVP	PPRDFHINLF
Medullasin	LI	HGIASFVR.G	GCASGLYPDA	FAPVA.....	....
Myeloblastin	II	QGIDSFVI.W	GCATRLFPDF	FTRVA.....	....
Complement C1S	PN.DKTKFYA	AGLVSWGP..	QCG.T..YGL	YTRVK.....	....
Complement C1R	PN.TD.RWVA	TGIVSWGI..	GCSRG..YGF	YTKVL.....	....
Factor X	RF.KDTYFV.	TGIVSWGE..	GCARKGKYGI	YTKVT.....	....
Factor IX	EV.EGTSFL.	TGIISWGE..	ECAMKGKYGI	YTKVS.....	....
Factor VII	HY.RGTWYL.	TGIVSWGQ..	GCATVGHFGV	YTRVS.....	....
Protein C	SF.HGTWFL.	VGLVSWGE..	GCGLLHNYGV	YTKVS.....	....
Thrombin	PF.NNRWYQ.	MGIVSWGE..	GCDRDGKYGF	YTHVF.....	....
u-PA	Q.G...RMTL	TGIVSWGR..	GCALKDKPGV	YTRVS.....	....
t-PA	D.G...RMTL	VGIISWGL..	GCGQKDVPGV	YTKVT.....	....
Factor XII	Q.AAERRLTL	QGIISWGS..	GCGDRNKPGV	YTDVA.....	....
Apolipoprotein A	....KDKYIL	QGVTSWG..L	GCARPNKPGV	YARVS.....	....
Plasmin	....KDKYIL	QGVTSWG..L	GCARPNKPGV	YVRVS.....	....
Hepsin	SISRTPRWRL	CGIVSWG..T	GCALAQKPGV	YTKVS.....	....
Elastase IIIa	E...DGGWQV	HGVTSFVSAF	GCNFIWKPTV	FTRVS.....	....
Elastase IIIb	E...DGGWQV	HGVTSFVSAF	GCNTRRKPTV	FTRVS.....	....
Elastase IIa	S...DGRWQV	HGIVSFGSRL	GCNYYHKPSV	FTRVS.....	....
Elastase IIb	S...DGRWEV	HGIGSLTSVL	GCNYYYKPSI	FTRVS.....	....
Chymotrypsin B	K...DGAWTL	VGIVSWGSDT	CST..SSPGV	YARVT.....	....
Alpha Tryptase	....NGTWLQ	AGVVSWDE..	GCAQPNRPGI	YTRVT.....	....
Beta Tryptase	....NGTWLQ	AGVVSWGE..	GCAQPNRPGI	YTRVT.....	....
Factor XI	....NEVWHL	VGITSWGE..	GCAQRERPGV	YTNVV.....	....
Plasma Kallikrein	....NGMWRL	VGITSWGE..	GCARREQPGV	YTKVA.....	....
Acrosin	S..KESAYVV	VGITSWG..V	GCALAKRPGI	YTATW.....	....
Trypsin I	........QL	QGVVSWGDG.	.CAQKNKPGV	YTKV.....	.........Y
Trypsin II	........EL	QGIVSWGYG.	.CAQKNRPGV	YTKV.....	.........Y
Trypsin III	........QL	QGVVSWGHG.	.CAWKNRPGV	YTKV.....	.........Y
Tissue Kallikrein 2	VL	QGITSWGPE.	PCALPEKPAV	YTKV.....	V
PSA	VL	QGITSWGSE.	PCALPERPSL	YTKV.....	V
Tissue Kallikrein 1	VL	QGVTSWGYV.	PCGTPNKPSV	AVRV.....	L
Granzyme B	........VA	QGIVSYGRNN	GMP....PRA	CTKVS.....	....
T-cell Granzyme	........VA	QGILSYGNKK	GTP....PGV	YIKVS.....	....
Cathepsin G	........VA	HGIVSYGKSS	GVP....PEV	FTRVS.....	....
Complement Factor D	VL	EGVVTSG.SR	VCGNRKKPGI	YTRVA.....	....
Granzyme A	........VF	RGVTSFGLEN	KCGDPRGPGV	YILLS.....	.........K
Complement Factor I	ANNVTYVW..	.GVVSWGE..	NCGKPEFPGV	YTKVA.....	....

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                        301                                                 350
Complement Factor B     QVLPWLKEKL QDEDLGFL.. ..... ..... .....
Complement C2           RMQPWLRQHL .GDVLNFLPL ..... ..... .....
Medullasin              QFVNWIDSII QRSEDNPCPH PRDPDPASRT H..... .....
Myeloblastin            LYVDWIRSTL RRVEAKGRP. ..... ..... .....
Complement C1S          NYVDWIMKTM QENSTPRED. ..... ..... .....
Complement C1R          NYVDWIKKEM EEED..... ..... ..... .....
Factor X                AFLKWIDRSM KTRGLPKAKS HAPEVITSSP LK..... .....
Factor IX               RYVNWIKEKT KLT..... ..... ..... .....
Factor VII              QYIEWLQKLM RSEPRPGVLL RAPFP..... ..... .....
Protein C               RYLDWIHGHI RDKEAPQKSW AP..... ..... .....
Thrombin                RLKKWIQKVI DQFGE..... ..... ..... .....
u-PA                    HFLPWIRSHT KEENGLAL.. ..... ..... .....
t-PA                    NYLDWIRDNM RP..... ..... ..... .....
Factor XII              YYLAWIREHT VS..... ..... ..... .....
Apolipoprotein A        RFVTWIEGMM RNN..... ..... ..... .....
Plasmin                 RFVTWIEGVM RNN..... ..... ..... .....
Hepsin                  DFREWIFQAI KTHSEASGMV TQL..... ..... .....
Elastase IIIa           AFIDWIEETI ASH..... ..... ..... .....
Elastase IIIb           AFIDWIEETI ASH..... ..... ..... .....
Elastase IIa            NYIDWINSVI ANN..... ..... ..... .....
Elastase IIb            NYNDWINSVI ANN..... ..... ..... .....
Chymotrypsin B          KLIPWVQKIL AAN..... ..... ..... .....
Alpha Tryptase          YYLDWIHHYV PKKP..... ..... ..... .....
Beta Tryptase           YYLDWIHHYV PKKP..... ..... ..... .....
Factor XI               EYVDWILEKT QAV..... ..... ..... .....
Plasma Kallikrein       EYMDWILEKT QSSDGKAQMQ SPA..... ..... .....
Acrosin                 PYLNWIASKI GSNALRMIQS ATPPPPTTRP PPIRPPFSHP ISAHLPWYFQ
Trypsin I               NYVKWIKNTI AANS..... ..... ..... .....
Trypsin II              NYVDWIKDTI AANS..... ..... ..... .....
Trypsin III             NYVDWIKDTI AANS..... ..... ..... .....
Tissue Kallikrein 2     HYRKWIKDTI AANP..... ..... ..... .....
PSA                     HYRKWIKDTI VANP..... ..... ..... .....
Tissue Kallikrein 1     SYVKWIEDTI AENS..... ..... ..... .....
Granzyme B              SFVHWIKKTM KRY..... ..... ..... .....
T-cell Granzyme         HFLPWIKRTM KRL..... ..... ..... .....
Cathepsin G             SFLPWIRTTM RSFKLLDQME TPL..... ..... .....
Complement Factor D     TYAAWIDHVL ..... ..... ..... .....
Granzyme A              KHLNWIIMTI KGAV..... ..... ..... .....
Complement Factor I     NYFDWISYHV GRPFISQYNV ..... ..... .....

                        351                                                 400
Acrosin                 PPPRPLPPRP PAAQPPPPPS PPPPPPPPAS PLPPPPPPPP PTPSSTTKLP
                        401                                         442
Acrosin                 QGLSFAKRLQ QLIEVLKGKT YSDGKNHYDM ETTELPELTS TS
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CLAIMS:

1. An endopeptidase of the chymotrypsin superfamily of serine proteases or a precursor of such an endopeptidase, which has been modified so as to exhibit resistance to serine protease inhibitors, characterised in that the modification comprises the mutation of one or more residues in close spacial proximity (other than sequential proximity) to a site of interaction between the protease and a cognate protease inhibitor.

2. A serine protease as claimed in claim 1, comprising plasmin, t-PA, u-PA, trypsin, chymotrypsin, granzyme, elastase, acrosin, tonin, myeloblastin, prostate-specific antigen (PSA), λ-renin, tryptase, snake venom serine proteases, adipsin, protein C, cathepsin G, complement components C1R, C1S and C2, complement factors B, D and I, chymase, hepsin, medullasin or proteins of the blood coagulation cascade including kallikrein, thrombin and factors VIIa, IXa, Xa, XIa and XIIa.

3. A serine protease as claimed in claim 1 or claim 2 which has a mutation in a region corresponding to residues 17-20, 44-54, 62, 154, 158, or 198-213 of the protease domain of plasmin (using the numbering of SEQ ID No 2).

4. A plasmin having a mutation as defined in claim 3.

5. A plasmin as claimed in claim 4, which has one or more of the following mutations: Glu-62 to Lys or Ala, Ser-17 to Leu, Arg 19 to Glu or Ala, or Glu-45 to Lys, Arg or Ala, (where the numbering is that of SEQ ID No 2).

6. A serine protease precursor as claimed in claim 1, which, when cleaved, forms a serine protease as claimed in any one of claims 2 to 5.

7. A serine protease precursor as claimed in claim 6, which is plasminogen or a plasminogen analogue.

8. A serine protease precursor as claimed in claim 7, wherein the plasminogen analogue is cleavable by an enzyme involved in blood clotting to produce active plasmin.

9. A process for the preparation of a serine protease or serine protease precursor as claimed in any one of claim 1 to 8, the process comprising coupling together successive amino acid residues and/or ligating oligopeptides.

10. A nucleic acid encoding a modified serine protease or serine protease precursor as claimed in any one of claims 1 to 8.

11. Nucleic acid as claimed in claim 10 which is a vector, for example, a plasmid, cosmid or phage.

12. Nucleic acid as claimed in claim 11 comprising a first nucleic acid sequence coding for the modified serine protease, operatively linked to a second nucleic acid sequence containing a strong promoter and enhancer sequence derived from human cytomegalovirus, a third nucleic acid sequence encoding a polyadenylation sequence derived from SV40 and a fourth nucleic acid sequence coding for a selectable marker expressed from an SV40 promoter and having an additional SV40 polyadenylation signal at the 3' end of the selectable marker sequence.

13. A process for the preparation of nucleic acid as claimed in any one of claims 10 to 12, the process comprising coupling together successive nucleotides and/or ligating oligo- and/or poly-nucleotides.

14. A cell transformed or transfected by nucleic acid as claimed in any one of claims 10 to 12.

15. A cell as claimed in claim 14 comprising a prokaryotic cell such as a bacterial cell, for example E. coli.

16. A cell as claimed in claim 14, which is a eukaryotic cell, for example a yeast cell such as Sacchromyces cerevisiae or Pichia pastoris or a mammalian cell such as a Chinese hamster ovary (CHO) cell, mouse myeloma cell, COS cell, HeLa cell, 293 cell, BHK cell, melanoma cell, mouse L cell, human hepatoma cell, mouse fibroblast or mouse NIH 3T3 cell.

17. A modified serine protease or serine protease precursor as claimed in any one of claims 1 to 8 and which has fibrinolytic, thrombolytic, antithrombotic or prothrombotic properties for use in medicine.

18. The use of a serine protease or precursor as claimed in any one of claims 1 to 8 and which has fibrinolytic, thrombolytic, antithrombotic or prothrombotic properties, in the preparation of an agent for the treatment and/or prophylaxis of diseases or conditions mediated by blood clotting.

19. A pharmaceutical or veterinary composition comprising one or more modified serine proteases as

claimed in any one of claims 1 to 8 together with a pharmaceutically and/or veterinarily acceptable carrier.

20. A eukaryotic host cell transfected or transformed with a first DNA sequence encoding a serpin-resistant serine proteases with their cognate inhibitor, and an additional DNA sequence encoding the cognate inhibitor.

21. A host cell as claimed in claim 20 where antiplasmin resistant plasminogen is co-expressed with antiplasmin

22. Antiplasmin-resistant plasminogen produced by the method of claim 21.

Figure 1 - Human Serine Proteases

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                    1                                                  50
Complement Factor B WEHRKGTDYH KQPWQAKISV IRPSKGH..E SCMGAVVSEY FVLTAAHCF.
      Complement C2 GVGNMSANAS DQERTPWHVT IKP.KSQ..E TCRGALISDQ WVLTAAHCF.
         Medullasin IVGGRRARPH AWPFMVSLQL R...GG...H FCGATLIAPN FVMSAAHCV.
        Myeloblastin .......... ....MASLQM RGNPGS...H FCGGTLIHPS FVLTAAHCL.
     Complement C1S IIGGSDADIK NFPWQVFF.. ..D.NP.... WAGGALINEY WVLTAAHVV.
     Complement C1R IIGGQKAKMG NFPWQVFT.. ..NIHG.... RGGGALLGDR WILTAAHTL.
           Factor X IVGGQECKDG ECPWQALLI. NEENEG.... FCGGTILSEF YILTAAHCL.
          Factor IX VVGGEDAKPG QFPWQVVL.. NGKVDA.... FCGGSIVNEK WIVTAAHCV.
         Factor VII IVGGKVCPKG ECPWQVLLL. VNGAQ..... LCGGTLINTI WVVSAAHCF.
          Protein C LIDGKMTRRG DSPWQVVLL. DSKKKL.... ACGAVLIHPS WVLTAAHCM.
          Thrombin IVEGSDAEIG MSPWQVMLFR KSPQEL.... LCGASLISDR WVLTAAHCLL
              u-PA IIGGEFTTIE NQPWFAAIYR RH.RGGSVTY VCGGSLMSPC WVISATHCF.
              t-PA IKGGLFADIA SHPWQAAIFA KHRRSPGERF LCGGILISSC WILSAAHCF.
         Factor XII VVGGLVALRG AHPYIAALYW GHS..... FCAGSLIAPC WVLTAAHCL.
Apolipoprotein A IVGGCVAHPH SWPWQVSL.R .TRFGK...H FCGGTLISPE WVLTAAHCL.
            Plasmin VVGGCVAHPH SWPWQVSL.R .TRFGM...H FCGGTLISPE WVLTAAHCL.
              Hepsin IVGGRDTSLG RWPWQVSL.R .YD.GA...H LCGGSLLSGD WVLTAAHCF.
     Elastase IIIa VVHGEDAVPY SWPWQVSL.Q .YEKSGSFYH TCGGSLIAPD WVVTAGHCI.
     Elastase IIIb VVNGEDAVPY SWPWQVSL.Q .YEKSGSFYH TCGGSLIAPD WVVTAGHCI.
      Elastase IIa VVGGEEARPN SWPWQVSL.Q .YSSNGKWYH TCGGSLIANS WVLTAAHCI.
      Elastase IIb MLGGEEARPN SWPWQVSL.Q .YSSNGQWYH TCGGSLIANS WVLTAAHCI.
    Chymotrypsin B IVNGEDAVPG SWPWQVSL.Q .DKTG...FH FCGGSLISED WVVTAAHCG.
     Alpha Tryptase IVGGQEAPRS KWPWQVSL.R .VR.DRYWMH FCGGSLIHPQ WVLTAAHCL.
      Beta Tryptase IVGGQEAPRS KWPWQVSL.R .VH.GPYWMH FCGGSLIHPQ WVLTAAHCV.
           Factor XI IVGGTASVRG EWPWQVTL.H .TT.SPTQRH LCGGSIIGNQ WILTAAHCF.
  Plasma Kallikrein IVGGTNSSWG EWPWQVSL.Q .VK.LTAQRH LCGGSLIGHQ WVLTAAHCF.
             Acrosin IVGGKAAQRG AWPWMVSL.Q IFRYNSHRYH TCGGSLLNSR WVLTAAHCF.
           Trypsin I IVGGYNCEEN SVPYQVSL.. ..NS.G..YH FCGGSLINEQ WVVSAGHCY.
          Trypsin II IVGGYICEEN SVPYQVSL.. ..NS.G..YH FCGGSLISEQ WVVSAGHCY.
         Trypsin III IVGGYTCEEN SLPYQVSL.. ..NS.G..SH FCGGSLISEQ WVVSAAHCY.
Tissue Kallikrein 2 IVGGWECEKH SQPWQVAV.. ..YSHG..WA HCGGVLVHPQ WVLTAAHCL.
             PSA IVGGWECEKH SQPWQVLV.. ..ASRG..RA VCGGVLVHPQ WVLTAAHCI.
Tissue Kallikrein 1 IVGGWECEQH SQPWQAAL.. ..YHFS..TF QCGGILVHRQ WVLTAAHCI.
         Granzyme B IIGGHEAKPH SRPYMAYL.M IWDQKS..LK RCGGFLIQDD FVLTAAHCW.
    T-cell Granzyme IIGGHEAKPH SRPYMAFV.Q FLQEKS..RK RCGGILVRKD FVLTAAHCQ.
         Cathepsin G IIGGRESRPH SRPYMAYL.Q IQSPAG..QS RCGGFLVRED FVLTAAHCW.
Complement Factor D ILGGREAEAH ARPYMASV.Q L...NG..AH LCGGVLVAEQ WVLSAAHCL.
         Granzyme A IIGGNEVTPH SRPYMVLL.S L...DR..KT ICAGALIAKD WVLTAAHC..
Complement Factor I IVGGKRAQLG DLPWQVAIKD ASGIT..... .CGGIYIGGC WILTAAHCL.
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                    51                                                  100
Complement Factor B ...TVDDKEH SI.KVSVGGE K....RDLEI EVVLFHPNYN INGKKEAGIP
      Complement C2 ...R.DGNDH SLWRVNVGDP KSQWGKELLI EKAVISPGFD VFAKKNQGIL
        Medullasin .....ANVNV RAVRVVLGAH NLSRREPTRQ VFAVQRIFEN GYDPVNLL..
       Myeloblastin .....RDIPQ RLVNVVLGAH NVRTQEPTQQ HFSVAQVFLN NYDAENKL..
  Complement C1S .......EGN REPTMYVGST SVQTSRLAKS KMLTPEHVFI HPGWKLLEVP
  Complement C1R YPKEHEAQSN ASLDVFLGHT NVEE..LMKL GNHPIRRVSV HPDYR....Q
         Factor X ....YQAK.. .RFKVRVGDR NTEQEEGG.E AVHEVEVVIK HNRF......
        Factor IX ....ETGV.. .KITVVAGEH NIEETEHT.E QKRNVIRIIP HHNYNA....
       Factor VII ....DKIKNW RNLIAVLGEH DLSEHDGD.E QSRRVAQVII PSTYVP....
        Protein C ....DESK.. .KLLVRLGEY DLRRWEKW.E LDLDIKEVFV HPNY......
       Thrombin YPPWDKNFTE NDLLVRIGKH SRTRYERNIE KISMLEKIYI HPRYNW....
          u-PA .IDYPKKE.. .DYIVYLGRS RLNSNTQGEM KF........ ..EVENLILH
          t-PA .QERFPPH.. .HLTVILGRT YRVVPGEEEQ KF........ ..EVEKYIVH
      Factor XII .QDRPAPE.. .DLTVVLGQE RRNHSCEPCQ TL........ ..AVRSYRLH
Apolipoprotein A .K..KSSRP. SSYKVILGAH QEV...NLES HV.....QE. ..IEVSRLFL
         Plasmin .E..KSPRP. SSYKVILGAH QEV...NLEP HV.....QE. ..IEVSRLFL
          Hepsin .P..ERNRVL SRWRVFAGAV AQASPHGLQL GV.....QA. ..VVYHGGYL
    Elastase IIIa .S..RD.... LTYQVVLGEY NLAVKEGPEQ VI.....PI. ..NSEELFVH
    Elastase IIIb .S..SS.... RIYQVVLGEY DRAVKEGPEQ VI.....PI. ..NSGDLFVH
     Elastase IIa .S..SS.... RTYRVGLGRH NLYVAESGSL AV.....SV. ....SKIVVH
     Elastase IIb .S..SS.... RIYRVMLGQH NLYVAESGSL AV.....SV. ....SKIVVH
  Chymotrypsin B .V..RT.... SDV.VVAGEF DQGSDEENIQ VL.....KI. ....AKVFKN
    Alpha Tryptase .G..PDVKDL ATLRVN.SGT HLYYQDQLLP VS.....RI. ..MVHPQFYI
     Beta Tryptase .G..PDVKDL AALRVQLREQ HLYYQDQLLP VS.....RI. ..IVHPQFYT
         Factor XI .YGVESPKIL RVYSGILNQS EIKEDTSFFG VQ.....EI. ..IIHDQYKM
  Plasma Kallikrein .DGLPLQDVW RIYSGILNLS DITKDTPFSQ IK.....EI. ..IIHQNYKV
           Acrosin .VGKNNVHD. ..WRLVFGAK EITYGNNKPV KA.....PLQ ERYVEKIIIH
         Trypsin I .....KSRI. ...QVRLGEH NIEVLEGNEQ F.INAAKIIR HPQYDRKTLN
        Trypsin II .....KSRI. ...QVRLGEH NIEVLEGNEQ F.INAAKIIR HPKYNSRTLD
       Trypsin III .....KTRI. ...QVRLGEH NIKVLEGNEQ F.INAAKIIR HPKYNRDTLD
Tissue Kallikrein 2 .....KKNS. ...QVWLGRH NLFEPEDTGQ R.VPVSHSFP HPLYNMSLLK
               PSA .....RNKS. ...VILLGRH SLFHPEDTGQ V.FQVSHSFP HPLYDMSLLK
Tissue Kallikrein 1 .....SDNY. ...QLWLGRH NLFDDENTAQ F.VHVSESFP HPGFNMSLLE
       Granzyme B .....GSSIN ....VTLGAH NIKEQEPTQQ F.IPVKRPIP HPAYNPKNFS
    T-cell Granzyme .....GSSIN ....VTLGAH NIKEQERTQQ F.IPVKRPIP HPAYNPKNFS
         Cathepsin G .....GSNIN ....VTLGAH NIQRRENTQQ H.ITARRAIR HPQYNQRTIQ
Complement Factor D .....EDAAD GKVQVLLGAT HLPQPEPXXX ITIEVLRAVP HPDSQPDTID
       Granzyme A .......NLN KRSQVILGAH SITREEPTKQ IML.VKKEFP YPCYDPATRE
Complement Factor I .....RASKT HRYQIWTTVV DWIHPDLKRI VIEYVDRIIF HENYNA....
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	101			150
Complement Factor B	EFY.....	DYDVALIKL.	....KNKLKY	GQTIRPICLP CTEGTTRALR
Complement C2	EFY.....	GDDIALLKL.	....AQKVKM	STHARPICLP CTMEANLALR
Meduliasin	.....	.NDIVILQL.	....NGSATI	NANVQVAQLP AQGR....RL
Myeloblastin	.....	.NDILLIQL.	....SSPANL	SASVTSVQLP QQDQ....PV
Complement C1S	E....GRTNF	DNDIALVRL.	....KDPVKM	GPTVSPICLP GTSSDYNLMD
Complement C1R	D....ESYNF	EGDIALLEL.	....ENSVTL	GPNLLPICLP DNDTFYDL..
Factor X	.....TKETY	DFDIAVLRL.	....KTPITF	RMNVAPACLP ERDWAESTL.
Factor IX	.....AINKY	NHDIALLEL.	....DEPLVL	NSYVTPICIA DKEYTN.IF.
Factor VII	.....G..TT	NHDIALLRL.	....HQPVVL	TDHVVPLCLP ERTFSERTL.
Protein C	.....SKSTT	DNDIALLHL.	....AQPATL	SQTIVPICLP DSGLAERELN
Thrombin	......RENL	DRDIALMKL.	....KKPVAF	SDYIHPVCLP DRETAASLLQ
u-PA	KDYSADTLAH	HNDIALLKIR	SK.EGRCAQP	SRTIQTICLP SMY..NDPQF
t-PA	KEFDDDT..Y	DNDIALLQLK	SD.SSRCAQE	SSVVRTVCLP P....ADLQL
Factor XII	EAFS..PVSY	QHDLALLRLQ	EDADGSCALL	SPYVQPVCLP SGA..ARP..
Apolipoprotein A	.....EPT	QADIALLKL.	...SRPAV.I	TDKVMPACLP SPD..YMVT.
Plasmin	.....EPT	RKDIALLKL.	...SSPAV.I	TDKVIPACLP SPN..YVVA.
Hepsin	PFRDPNSEEN	SNDIALVHL.	...SSPLP.L	TEYIQPVCLP AAG..QALV.
Elastase IIIa	PLWNRSCVAC	GNDIALIKL.	...SRSAQ.L	GDAVQLASLP PAG..DILP.
Elastase IIIb	PLWNRSCVAC	GNDIALIKL.	...SRSAQ.L	GDAVQLASLP PAG..DILP.
Elastase IIa	KDWNSNQISK	GNDIALLKL.	...ANPVS.L	TDKIQLACLP PAG..TILP.
Elastase IIb	KDWNSNQVSK	GNDIALLKL.	...ANPVS.L	TDKIQLACLP PAG..TILP.
Chymotrypsin B	PKF..SILTV	NNDITLLKL.	...ATPAR.F	SQTVSAVCLP SAD..DDFP.
Alpha Tryptase	.....IQT	GADIALLEL.	...EEPVN.I	SSRVHTVMLP PAS..ETFP.
Beta Tryptase	.....AQI	GADIALLEL.	...EEPVK.V	SSHVHTVTLP PAS..ETFP.
Factor XI	.....AES	GYDIALLKL.	...ETTVN.Y	TDSQRPICLP SKG..DRNV.
Plasma Kallikrein	.....SEG	NHDIALIKL.	...QAPLN.Y	TEFQKPICLP SKG..DTST.
Acrosin	EKYNS..ATE	GNDIALVEI.	...TPPIS.C	GRFIGPGCLP HFK..AGLP.
Trypsin I	N.....	..DIMLIKL.	...SSRA.VI	NARVSTISLP TAP..PAT..
Trypsin II	N.....	..DILLIKL.	...SSPA.VI	NSRVSAISLP TAP..PAA..
Trypsin III	N.....	..DIMLIKL.	...SSPA.VI	NARVSTISLP TAP..PAA..
Tissue Kallikrein 2	HQSLRPDEDS	SHDLMLLRL.	...SEPAK.I	TDVVKVLGLP TQE..PAL..
PSA	NRFLRPGDDS	SHDLMLLRL.	...SEPAE.L	TDAVKVMDLP TQE..PAL..
Tissue Kallikrein 1	NHTRQADEDY	SHDLMLLRL.	...TEPADTI	TDAVKVVELP TQE..PEV..
Granzyme B	N.....	..DIMLLQL.	...ERKAK.R	TRAVQPLRLP SNK..AQVK.
T-cell Granzyme	N.....	..DIMLLQL.	...ERKAK.W	TTAVRPLRLP SSK..AQVK.
Cathepsin G	N.....	..DIMLLQL.	...SRRVR.R	NRNVNPVALP RAQ..EGLR.
Complement Factor D	H.....	..DLLLLQL.	...SEKAT.L	GPAVRPLPWQ RVD..RDVA.
Granzyme A	G.....	..DLKLLQL.	...TEKAK.I	NKYVTILHLP KKG..DDVK.
Complement Factor I	.....GTY	QNDIALIEMK	KDGNKKDCEL	.....PRSIP ACVPWSPYLF

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                    151                                                 200
Complement Factor B LPPTTTCQQQ KEELLPAQDI KALFVSEEEK KLTRKEVYIK NGDKKGSC.E
     Complement C2  RPQGSTCRDH ENELLNKQSV PAHFVALNGS KL...NINLK MGVEWTSCAE
       Medullasin   GNGVQCLAMG WGLL..... GRNRGIASVL QELNVTV... ..VT......
     Myeloblastin   PHGTQCLAMG WGRV..... GAHDPPAQVL QELNVTV... ..VT......
  Complement C1S    GDL..GLISG WGRTEK.... ...RDRAVRL KAARLPV... ..APLRKCKE
  Complement C1R    GLM..GYVSG FGVMEE.... ...KI.AHDL RFVRLPV... ..ANPQACEN
         Factor X   MTQKTGIVSG FGRTHE.KGR QS.....TRL KMLEVPY... ..VDRNSCKL
       Factor IX    LKFGSGYVSG WGRVFH.KGR SA.....LVL QYLRVPL... ..VDRATCLR
      Factor VII    AFVRFSLVSG WGQLLD.RGA TA.....LEL MVLNVPR... ..LMTQDCLQ
       Protein C    QAGQETLVTG WGYHSS.REK EAKRNRTFVL NFIKIPV... ..VPHNECSE
        Thrombin    AGYK.GRVTG WGNLKETWTA NVGKGQPSVL QVVNLPI... ..VERPVCKD
           u-PA     G..TSCEITG FGKENS.... TDYLYPEQ.L KMTVVKL... ..ISHRECQQ
           t-PA     PDWTECELSG YGKHEA.... LSPFYSER.L KEAHVRL... ..YPSSRCTS
      Factor XII    SETTLCQVAG WGHQFE.... GAEEYASF.L QEAQVPF... ..LSLERCSA
Apolipoprotein A    ARTE.CYITG WGETQG.... TFG..TG.LL KEAQLLV... ..IENEVCNH
         Plasmin    DRTE.CFITG WGETQG.... TFG..AG.LL KEAQLPV... ..IENKVCNR
           Hepsin   DGKI.CTVTG WGNTQ..... YYGQQAG.VL QEARVPI... ..ISNDVCNG
     Elastase IIIa  NKTP.CYITG WGRLYT.... NGP.LPD.KL QQARLPV... ..VDYKHCSR
     Elastase IIIb  NETP.CYITG WGRLYT.... NGP.LPD.KL QEALLPV... ..VDYEHCSR
      Elastase IIa  NNYP.CYVTG WGRLQT.... NGA.VPD.VL QQGRLLV... ..VDYATCSS
      Elastase IIb  NNYP.CYVTG WGRLQT.... NGA.LPD.DL KQGRLLV... ..VDYATCSS
   Chymotrypsin B   AGTL.CATTG WGKTKY.... NANKTPD.KL QQAALPL... ..LSNAECKK
    Alpha Tryptase  PGMP.CWVTG WGDVDN.... DEPLPPPFPL KQVKVPI... ..MENHICDA
     Beta Tryptase  PGMP.CWVTG WGDVDN.... DERLPPPFPL KQVKVPI... ..MENHICDA
         Factor XI  IYTD.CWVTG WGYRKL.... RDKIQN..TL QKAKIPL... ..VTNEECQK
Plasma Kallikrein   IYTN.CWVTG WGFSKE.... KGEIQN..IL QKVNIPL... ..VTNEECQK
           Acrosin  RGSQSCWVAG WGYIEE.... KAP.RPSSIL MEARVDL... ..IDLDLCNS
        Trypsin I   .GTK.CLISG WGNTAS.... SGADYPD.EL QCLDAPV... ..LSQAKCEA
       Trypsin II   .GTE.SLISG WGNTLS.... SGADYPD.EL QCLDAPV... ..LSQAECEA
      Trypsin III   .GTE.CLISG WGNTLS.... FGADYPD.EL KCLDAPV... ..LREAECKA
Tissue Kallikrein 2 .GTT.CYASG WGSIEP.... EEFLRPR.SL QCVSLHL... ..LSNDMCAR
              PSA   .GTT.CYASG WGSIEP.... EEFLTPK.KL QCVDLHV... ..ISNDVCAQ
Tissue Kallikrein 1 .GST.CLASG WGSIEP.... ENFSFPD.DL QCVDLKI... ..LPNDECEK
       Granzyme B   PGQT.CSVAG WGQTAP.... LG.KHSH.TL QEVKMTV... ..QEDRKCES
    T-cell Granzyme PGQL.CSVAG WG.YVS.... MS.TLAT.TL QEVLLTV... ..QKDCQCER
       Cathepsin G  PGTL.CTVAG WGR.VS.... MR.RGTD.TL REVQLRV... ..QRDRQCLR
Complement Factor D PGTL.CDVAG WGIVNH.... AG.RRPD.SL QHVLLPV... ..LDRATCRL
       Granzyme A   PGTM.CQVAG WGRTHN.... SA.SWSD.TL REVNITI... ..IDRKVCND
Complement Factor I QPNDTCIVSG WGREKDNERV FSLQWGEVKL ISNCSKF... ..YGNRFYEK
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                    201                                                 250
Complement Factor B RDAQYAPGYD KVKDISEVVT PRFLCTGGVS PYADPNTCRG DSGGPLIVHK
     Complement C2  VVSQEKTMFP NLTDVREVVT DQFLCSGTQ. ..EDESPCKG ESGGAVFLER
       Medullasin   .......... ........SL CRRSNVCTLV RGRQAGVCFG DSGSPLVCNG
     Myeloblastin   .......... ........FF CRPHNICTFV PRRKAGICFG DSGGPLICDG
     Complement C1S VKVEKPTADA EAYVFTPNMI CAG....GEK G...MDSCKG DSGGAFAVQD
     Complement C1R WLRGKNRMD. ...VFSQNMF CAGH...PSL K...QDACQG DSGGVFAVRD
          Factor X  ....SSSFI. ....ITQNMF CAGY...DTK Q...EDACQG DSGGPHV..T
         Factor IX  ....STKFT. ....IYNNMF CAGF...HEG G...RDSCQG DSGGPHV..T
        Factor VII  ....QSRKVG DSPNITEYMF CAGY...SDG S...KDSCKG DSGGPHA..T
         Protein C  ....VMSNM. ....VSENML CAGI...LGD R...QDACEG DSGGPMV..A
          Thrombin  ....STRI.. ...RITDNMF CAGYKPDEGK R...GDACEG DSGGPFVMKS
              u-PA  PHYYGS.... ...EVTTKML CAADPQWKT. .....DSCQG DSGGPLVCSL
              t-PA  QHLLNR.... ...TVTDNML CAGDTRSGGP QANLHDACQG DSGGPLVCLN
         Factor XII PDVHGS.... ...SILPGML CAGFLEGGT. .....DACQG DSGGPLVCED
  Apolipoprotein A  YKY....... .........I CAEHLARGT. .....DSCQG DSGGPLVCFE
            Plasmin YEFLNG.... ...RVQSTEL CAGHLAGGT. .....DSCQG DSGGPLVCFE
              Hepsin ADFYGN.... ...QIKPKMF CAGYPEGGI. .....DACQG DSGGPFVCED
     Elastase IIIa   WNWWGS.... ...TVKKTMV CAG.GY.IR. .....SGCNG DSGGPLNCPT
     Elastase IIIb   WNWWGS.... ...SVKKTMV CAG.GD.IR. .....SGCNG DSGGPLNCPT
      Elastase IIa   SAWWGS.... ...SVKTSMI CAG.GDGVI. .....SSCNG DSGGPLNCQA
      Elastase IIb   SGWWGS.... ...TVKTNMI CAG.GDGVI. .....CTCNG DSGGPLNCQA
  Chymotrypsin B     S..WGR.... ...RITDVMI CAG.ASGV.. .....SSCMG DSGGPLVCQ.
    Alpha Tryptase   KYHLGAYTGD DVRIIRDDML CAG..NSQR. .....DSCKG DSGGPLVCKV
     Beta Tryptase   KYHLGAYTGD DVRIVRDDML CAG..NTRR. .....DSCQG DSGGPLVCKV
          Factor XI  RYR....... .GHKITHKMI CAGYREGGK. .....DACKG DSGGPLSCKH
  Plasma Kallikrein  RYQ....... .DYKITQRMV CAGYKEGGK. .....DACKG DSGGPLVCKH
            Acrosin  TQWYNG.... ...RVQPTNV CAGYPVGKI. .....DTCQG DSGGPLMCKD
          Trypsin I  ....S..... YPGKITSNMF CVGFLEGGK. .....DSCQG DSGGPVVCNG
         Trypsin II  ....S..... YPGKITNNMF CVGFLEGGK. .....DSCQG DSGGPVVSNG
        Trypsin III  ....S..... CPGKITNSMF CVGFLEGGK. .....DSWKR DSGGPVVCNG
Tissue Kallikrein 2  ....A..... YSEKVTEFML CAGLWTGGK. .....DTCGG DSGGPLVCNG
                PSA  ....V..... HPQKVTKFML CAGRWTGGK. .....STCSG DSGGPLVCNG
Tissue Kallikrein 1  ....A..... HVQKVTDFML CVGHLEGGK. .....DTCVG DSGGPLMCDG
          Granzyme B DLRHY..... YDSTIEL... CVGDPEIKK. .....TSFKG DSGGPLVCNK
   T-cell Granzyme   LFHGN..... YSRATEI... CVGDPKKTQ. .....TGFKG DSGGPLVCKD
         Cathepsin G IF.GS..... YDPRRQI... CVGDRRERK. .....AAFKG DSGGPLLCNN
Complement Factor D  YD..... .....VLRLM CAESNR..R. .....DSCKG DSGGPLVCGG
          Granzyme A RNHYN..... FNPVIGMNMV CAGSLRGGR. .....DSCNG DSGSPLLCEG
Complement Factor I  .......... .......EME CAGTYDGSI. .....DACKG DSGGPLVCMD
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                    251                                                300
Complement Factor B RS....RFIQ VGVISWGVVD VC...KNQKR QKQVP....A HARDFHINLF
      Complement C2 RF....RFFQ VGLVSWGLYN PCLGSADKNS RKRAPRSKVP PPRDFHINLF
         Medullasin ........LI HGIASFVR.G GCASGLYPDA FAPVA..... ..........
        Myeloblastin ........II QGIDSFVI.W GCATRLFPDF FTRVA..... ..........
  Complement C1S PN.DKTKFYA AGLVSWGP.. QCG.T..YGL YTRVK..... ..........
  Complement C1R PN.TD.RWVA TGIVSWGI.. GCSRG..YGF YTKVL..... ..........
          Factor X RF.KDTYFV. TGIVSWGE.. GCARKGKYGI YTKVT..... ..........
         Factor IX EV.EGTSFL. TGIISWGE.. ECAMKGKYGI YTKVS..... ..........
        Factor VII HY.RGTWYL. TGIVSWGQ.. GCATVGHFGV YTRVS..... ..........
        Protein C SF.HGTWFL. VGLVSWGE.. GCGLLHNYGV YTKVS..... ..........
         Thrombin PF.NNRWYQ. MGIVSWGE.. GCDRDGKYGF YTHVF..... ..........
            u-PA Q.G...RMTL TGIVSWGR.. GCALKDKPGV YTRVS..... ..........
            t-PA D.G...RMTL VGIISWGL.. GCGQKDVPGV YTKVT..... ..........
        Factor XII Q.AAERRLTL QGIISWGS.. GCGDRNKPGV YTDVA..... ..........
Apolipoprotein A ....KDKYIL QGVTSWG..L GCARPNKPGV YARVS..... ..........
           Plasmin ....KDKYIL QGVTSWG..L GCARPNKPGV YVRVS..... ..........
            Hepsin SISRTPRWRL CGIVSWG..T GCALAQKPGV YTKVS..... ..........
      Elastase IIIa E...DGGWQV HGVTSFVSAF GCNFIWKPTV FTRVS..... ..........
      Elastase IIIb E...DGGWQV HGVTSFVSAF GCNTRRKPTV FTRVS..... ..........
       Elastase IIa S...DGRWQV HGIVSFGSRL GCNYYHKPSV FTRVS..... ..........
       Elastase IIb S...DGRWEV HGIGSLTSVL GCNYYYKPSI FTRVS..... ..........
  Chymotrypsin B K...DGAWTL VGIVSWGSDT CST..SSPGV YARVT..... ..........
  Alpha Tryptase ....NGTWLQ AGVVSWDE.. GCAQPNRPGI YTRVT..... ..........
   Beta Tryptase ....NGTWLQ AGVVSWGE.. GCAQPNRPGI YTRVT..... ..........
         Factor XI ....NEVWHL VGITSWGE.. GCAQRERPGV YTNVV..... ..........
Plasma Kallikrein ....NGMWRL VGITSWGE.. GCARREQPGV YTKVA..... ..........
          Acrosin S..KESAYVV VGITSWG..V GCALAKRPGI YTATW..... ..........
        Trypsin I ........QL QGVVSWGDG. .CAQKNKPGV YTKV..... .........Y
       Trypsin II ........EL QGIVSWGYG. .CAQKNRPGV YTKV..... .........Y
      Trypsin III ........QL QGVVSWGHG. .CAWKNRPGV YTKV..... .........Y
Tissue Kallikrein 2 ........VL QGITSWGPE. PCALPEKPAV YTKV..... .........V
              PSA ........VL QGITSWGSE. PCALPERPSL YTKV..... .........V
Tissue Kallikrein 1 ........VL QGVTSWGYV. PCGTPNKPSV AVRV..... .........L
        Granzyme B ........VA QGIVSYGRNN GMP....PRA CTKVS..... ..........
    T-cell Granzyme ........VA QGILSYGNKK GTP....PGV YIKVS..... ..........
         Cathepsin G ........VA HGIVSYGKSS GVP....PEV FTRVS..... ..........
Complement Factor D ........VL EGVVTSG.SR VCGNRKKPGI YTRVA..... ..........
        Granzyme A ........VF RGVTSFGLEN KCGDPRGPGV YILLS..... .........K
Complement Factor I ANNVTYVW.. .GVVSWGE.. NCGKPEFPGV YTKVA..... ..........
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                    301                                                 350
Complement Factor B QVLPWLKEKL QDEDLGFL.. ..... ..... .....
     Complement C2  RMQPWLRQHL .GDVLNFLPL ..... ..... .....
       Medullasin   QFVNWIDSII QRSEDNPCPH PRDPDPASRT H..... .....
      Myeloblastin  LYVDWIRSTL RRVEAKGRP. ..... ..... .....
     Complement C1S NYVDWIMKTM QENSTPRED. ..... ..... .....
     Complement C1R NYVDWIKKEM EEED..... ..... ..... .....
          Factor X  AFLKWIDRSM KTRGLPKAKS HAPEVITSSP LK..... .....
         Factor IX  RYVNWIKEKT KLT..... ..... ..... .....
        Factor VII  QYIEWLQKLM RSEPRPGVLL RAPFP..... ..... .....
        Protein C   RYLDWIHGHI RDKEAPQKSW AP..... ..... .....
         Thrombin   RLKKWIQKVI DQFGE..... ..... ..... .....
            u-PA    HFLPWIRSHT KEENGLAL.. ..... ..... .....
            t-PA    NYLDWIRDNM RP..... ..... ..... .....
        Factor XII  YYLAWIREHT VS..... ..... ..... .....
  Apolipoprotein A  RFVTWIEGMM RNN..... ..... ..... .....
          Plasmin   RFVTWIEGVM RNN..... ..... ..... .....
           Hepsin   DFREWIFQAI KTHSEASGMV TQL..... ..... .....
     Elastase IIIa  AFIDWIEETI ASH..... ..... ..... .....
     Elastase IIIb  AFIDWIEETI ASH..... ..... ..... .....
      Elastase IIa  NYIDWINSVI ANN..... ..... ..... .....
      Elastase IIb  NYNDWINSVI ANN..... ..... ..... .....
    Chymotrypsin B  KLIPWVQKIL AAN..... ..... ..... .....
     Alpha Tryptase YYLDWIHHYV PKKP..... ..... ..... .....
      Beta Tryptase YYLDWIHHYV PKKP..... ..... ..... .....
         Factor XI  EYVDWILEKT QAV..... ..... ..... .....
  Plasma Kallikrein EYMDWILEKT QSSDGKAQMQ SPA..... ..... .....
           Acrosin  PYLNWIASKI GSNALRMIQS ATPPPPTTRP PPIRPPFSHP ISAHLPWYFQ
         Trypsin I  NYVKWIKNTI AANS..... ..... ..... .....
        Trypsin II  NYVDWIKDTI AANS..... ..... ..... .....
       Trypsin III  NYVDWIKDTI AANS..... ..... ..... .....
Tissue Kallikrein 2 HYRKWIKDTI AANP..... ..... ..... .....
              PSA   HYRKWIKDTI VANP..... ..... ..... .....
Tissue Kallikrein 1 SYVKWIEDTI AENS..... ..... ..... .....
       Granzyme B   SFVHWIKKTM KRY..... ..... ..... .....
    T-cell Granzyme HFLPWIKRTM KRL..... ..... ..... .....
       Cathepsin G  SFLPWIRTTM RSFKLLDQME TPL..... ..... .....
Complement Factor D TYAAWIDHVL ..... ..... ..... .....
       Granzyme A   KHLNWIIMTI KGAV..... ..... ..... .....
Complement Factor I NYFDWISYHV GRPFISQYNV ..... ..... .....

                    351                                                 400
           Acrosin  PPPRPLPPRP PAAQPPPPPS PPPPPPPPAS PLPPPPPPPP PTPSSTTKLP
                    401                                         442
           Acrosin  QGLSFAKRLQ QLIEVLKGKT YSDGKNHYDM ETTELPELTS TS
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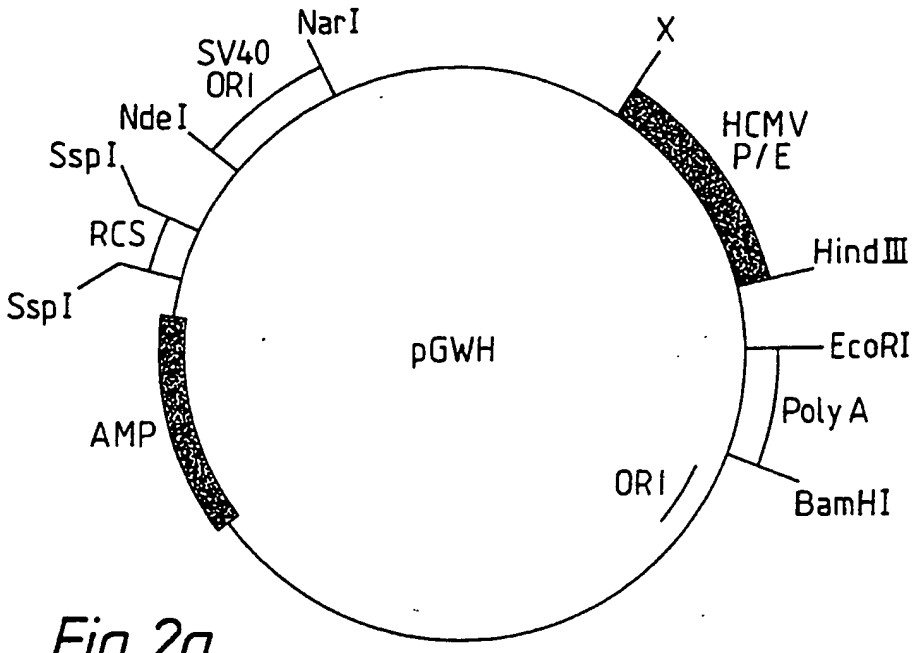
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ORI
NdeI
SspI
RCS
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EcoRI
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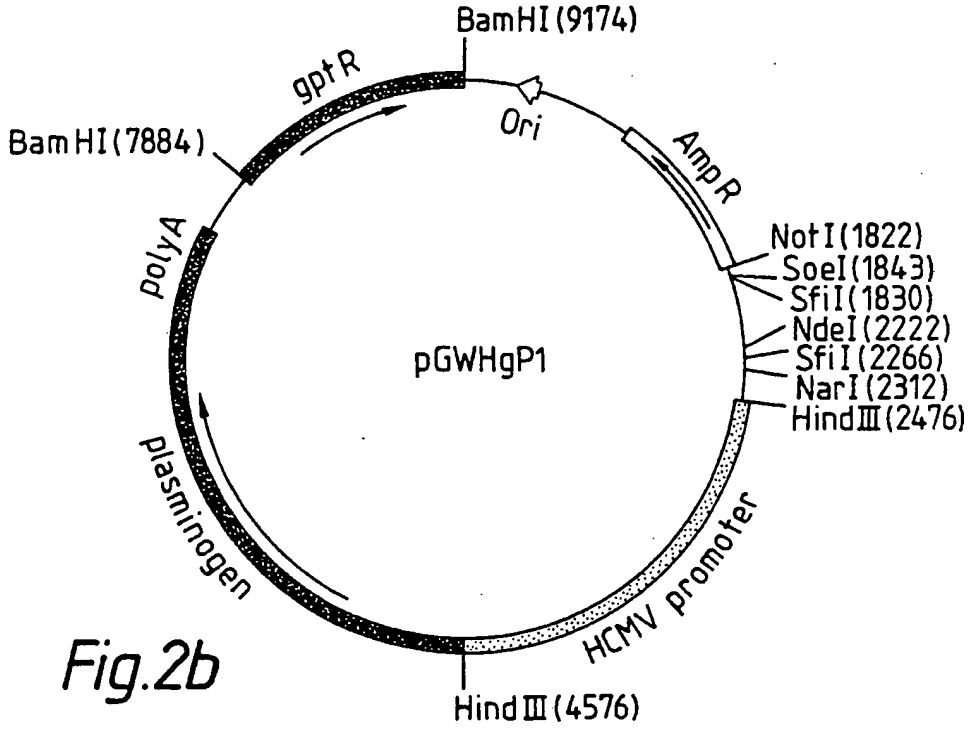
ORI
pGWH

Fig.2a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gptR
Ori
AmpR
BamHI (7884)
polyA
NotI (1822)
SoeI (1843)
SfiI (1830)
NdeI (2222)
SfiI (2266)
NarI (2312)
HindIII (2476)
pGWHgP1
plasminogen
HCMV promoter
HindIII (4576)

Fig.2b

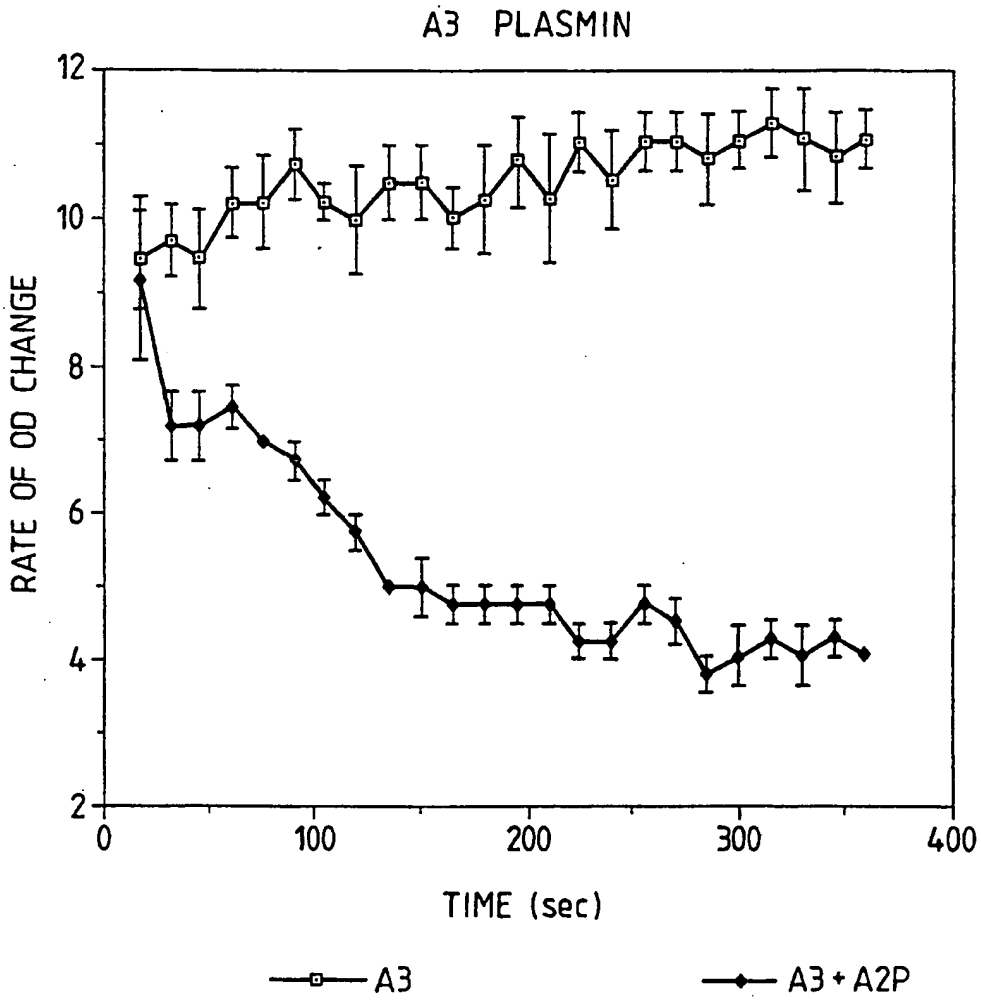
A3 PLASMIN
RATE OF OD CHANGE
12
10
8
6
4
2
0
100
200
300
400
TIME (sec)
A3
A3 + A2P

Fig. 3

```
                1                                                  50
Antiplasmin     MALLWGLLVL SWSCLQGPCS VFSPVSAMEP LGRQLTSGPN QEQVSPLTLL

               51                                                 100
Antiplasmin     KLGNQEPGGQ TALKSPPGVC SRDPTPEQTH RLARAMMAFT ADLFSLVAQT
  Ovalbumin     ---------- ---------- ---------G SIGAASMEFC FDVFKELKVH

              101                                                 150
Antiplasmin     STCPNLILSP LSVALALSHL ALGAQNHTLQ RLQQVLHAGS GP--------
  Ovalbumin     HANENIFYCP IAIMSALAMV YLGAKDSTRT QINKVVRFDK LPGFGDSIEA

              151                                                 200
Antiplasmin     ---------- CLPHLLSRLC QDLGPGAFRL AARMYLQKGF PIKEDFLEQS
  Ovalbumin     QCGTSVNVHS SLRDILNQIT KPNDVYSFSL ASRLYAEERY PILPEYLQCV

              201                                                 250
Antiplasmin     EQLF--GAKP VSLTGKQEDD LANINQWVKE ATEGKIQEFL S--GLPEDTV
  Ovalbumin     KELYRGGLEP INFQTAADQA RELINSWVES QTNGIIRNVL QPSSVDSQTA

              251                                                 300
Antiplasmin     LLLLNAIHFQ GFWRNKFDPS LTQRDSFHLD EQFTVPVEMM -QARTYPLRW
  Ovalbumin     MVLVNAIVFK GLWEKAFKDE DTQAMPFRVT EQESKPVQMM YQIGLFRVAS
```

Fig. 4

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                301                                                 350
Antiplasmin     FLLEQPEIQV AHFPFKNNMS FVVLVPTHFE WNVSQVLANL SWDTLHPPLV
 Ovalbumin      MASEKMKILE LPF-ASGTMS MLVLLPDEVS -GLEQLESII NFEKLTEWTS

                351                                                 400
Antiplasmin     WE----RPTK VRLPKLYLKH QMDLVATLSQ LGLQELF-QA PDLRGIS-EQ
 Ovalbumin      SNVMEERKIK VYLPRMKMEE KYNLTSVLMA MGITDVFSSS ANLSGISSAE

                401                                                 450
  BBTI Loop                                    P CKARII
Antiplasmin     SLVVSGVQHQ STLELSEVGV EAAAATSIAM SRMSLSS-FS VNRPFLFFIF
 Ovalbumin      SLKISQAVHA AHAEINEAGR EVVGSAEAGV DAASVSEEFR ADHPFLFCIK

                451                                                 500
Antiplasmin     EDTTGLPLFV GSVRNPNPSA PRELKEQQDS PGNKDFLQSL KGFPRGDKLF
 Ovalbumin      HIATNAVLFF GRCVSP

                501                 521
Antiplasmin     GPDLKLVPPM EEDYPQFGSP K
```

Fig.4 (cont.)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 93/01632

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/57 C12N15/58 C12N9/64 C12N9/66 C12N9/68
C12N9/72 C12N9/74 C12N9/76 A61K37/547 C12N1/21
C12N1/19 C12N5/10 C12N15/15

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 06203 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM) 16 April 1992 see the whole document ---	1-3,6, 10,11, 13-19
X	WO,A,90 10649 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM) 20 September 1990 cited in the application see the whole document ---	1-3,6, 10,11, 13-19
X	EP,A,0 381 331 (INTEGRATED GENETICS) 8 August 1990 see claims; table 4 --- -/--	1-3,6, 10,11, 13-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 November 1993	16. 12. 93

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Van der Schaal, C

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,91 09118 (BRITISH BIO-TECHNOLOGY LIMITED) 27 June 1991 cited in the application see the whole document	1-19

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. nal Application No
PCT/GB 93/01632

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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WO-A-9010649	20-09-90	AU-B-	637791	10-06-93
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		EP-A-	0462207	27-12-91
		JP-T-	4504952	03-09-92
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		JP-A-	2295483	06-12-90
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		AU-A-	6965691	18-07-91
		CA-A-	2069105	08-06-91
		EP-A-	0502968	16-09-92
		EP-A-	0504241	23-09-92
		WO-A-	9109125	27-06-91
		JP-T-	5502374	28-04-93
		JP-T-	5502375	28-04-93